1033 Issued October 26, 1907.

U. S. DEPARTMENT OF AGRICULTURE.

OFFICE OF EXPERIMENT STATIONS—BULLETIN 194.

A. C. TRUE, Director.

A REVIEW OF INVESTIGATIONS IN SOIL BACTERIOLOGY.

BY

EDWARD B. VOORHEES, D. Sc.,

Director, New Jersey Agricultural Experiment Stations,

AND

JACOB G. LIPMAN, Ph. D.,

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THE OFFICE OF EXPERIMENT STATIONS.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Office of Experiment Stations,
Washington, D. C., August 31, 1907.

Sir: I have the honor to transmit herewith, and to recommend for publication as a bulletin of this Office, a review of investigations in soil bacteriology, by Edward B. Voorhees, D. Sc., director, and Jacob G. Lipman, Ph. D., soil chemist and bacteriologist, of the New Jersey Experiment Stations. This review covers the more important contributions to the subject of soil bacteriology up to the end of 1906.

This Department and a few of the experiment stations have made important contributions to the subject of soil bacteriology, particularly in the field of fixation of nitrogen by micro-organisms in symbiosis with leguminous plants. A study of the subject in its broader aspects, including not only symbiotic but nonsymbiotic fixation of nitrogen; nitrification and other biological processes of transforming nitrogen compounds in the soil; the rôle of micro-organisms in rendering available the mineral constituents of plant food in the soil; and a number of other processes having an intimate bearing upon soil fertility and productiveness, has, however, been limited to the work of a comparatively small number of investigators in this country. On the other hand there has been rapid development along these lines in other countries, and enough has been accomplished to indicate that the biological processes are of equal importance with chemical and physical processes in the soil, and therefore deserve equal consideration in studies of soil fertility.

It is hoped that the publication of this review may serve to call attention to the progress already made, to indicate the possibilities of the subject, and to stimulate further and broader inquiry in this important field in this country.

Respectfully,

A. C. True,
Director.

Hon. James Wilson,
Secretary of Agriculture.

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A REVIEW OF INVESTIGATIONS IN SOIL BACTERIOLOGY.

INTRODUCTION.

Soil fertility, broadly interpreted, denotes the crop-producing power of any soil under given climatic conditions, and is itself the resultant of many forces often opposed to one another. It is no easy task to establish the correlation of these several forces, and to assign to each its true position as a factor in the creation of plant substance. We do know that suitable moisture and temperature conditions are indispensable not only for the absorption and assimilation of the plant food in the soil, but also for the formation of this plant food. Granting, however, that the moisture and temperature conditions are suitable, our inquiry is at once directed toward the ways and means whereby the soil is made to yield nourishment to the plants growing upon it. Obviously, an intimate knowledge of the ways and means of plant-food production is, aside from its theoretical interest, of great practical concern, a fact which is exemplified by the benefits derived by our agricultural industries from the researches of the agricultural chemist. It was through the investigations of the latter that we have learned to know the concrete meaning of the term plant food, and have learned also to apply this knowledge to the incalculable advantage of society. But as the rapidly accumulating experimental data broadened his horizon, the agricultural chemist was the first to recognize that chemical methods alone were wholly inadequate for a clear comprehension of the complicated processes occurring in arable soils. The keen insight of Hilgard[a] revealed the vast possibilities that lay in the joint application of chemical and physical methods in the study of soil-fertility problems, and the splendid contributions of soil physics to scientific agriculture bear testimony to the soundness of his judgment.

It was the no less keen discernment of another agricultural chemist, Berthelot,[b] that largely contributed to a better appreciation of

[a] Amer. Jour. Sci., 106 (1873), p. 288; U. S. Dept. Agr., Bur. Chem. Bul. 38, p. 60; California Sta. Rpt. 1892–1894, p. 100.

[b] Chimie Vegetale et Agricole. Paris: Masson et Cie., 1899, Vol. I, p. 9.

soil bacteriological activities as a factor in plant production. His experiments, dealing in the main with specific bacteriological processes, just as the work of Schloesing and Müntz,[a] Warington,[b] and Winogradski,[c] of Dehérain and Maquenne,[d] of Gayon and Dupetit,[e] or of Hellriegel and Wilfarth,[f] dealt with specific processes, furnished, none the less, a strong argument for a more systematic study of soil bacteria in general, and led gradually to the recognition of bacteriological methods as a valuable aid in the study of soil problems. Henceforth the soil chemist, soil physicist, and soil bacteriologist, working in harmony, must each do his share in the solution of these problems.

Soil bacteria were probably among the earliest living organisms on our planet. In a communication of December 29, 1879, Van Tieghem[g] stated that he had found fossil bacteria in the fossilized remains of ancient plants. In the marshes of the carboniferous era, he wrote, as in the swamps of to-day, the same plants suffered in the same portions of their tissues the same decomposition by the same agent. Then, as now, *Bacillus amylobacter* was the great destroyer of plant organisms, and the butyric fermentation which it caused in cellulose as well as in all the other substances that it used as nourishment seems to have been one of the most universal phenomena in the world of organized beings. Some years later Renault[h] demonstrated the presence of fossilized bacteria, in the beds of the Permian period, in the upper, middle, and lower strata of the carboniferous era, in carboniferous limestone, and in strata of the Devonian age. It would seem thus that long ages before man himself came to this earth there already existed microscopic forms of life which found food and energy in the destruction of organic substances. We shall never know of the countless species that came and vanished with the changing centuries; we shall never know more intimately of the long process of selection, of elimination, and of adaptation to a new environment. We can only note in our own day those stages of transition with which we are more or less familiar and wonder how the striking generic and specific differences were established. Somewhere in the course of evolution there came into

[a] Compt. Rend. Acad. Sci. [Paris], 80 (1875), p. 1250; 84 (1877), p. 301; 85 (1877), p. 1018.

[b] Jour. Chem. Soc. [London], 59 (1891), p. 484; U. S. Dept. Agr., Office of Experiment Stations Bul. 8, 1892.

[c] Ann. Inst. Pasteur, 4 (1890), pp. 213, 257, 760; 5 (1891), pp. 92, 577.

[d] Ann. Agron., 9 (1883), p. 6.

[e] Recherches sur la reduction des nitrates par les infiniment petits. Bordeaux: G. Gounouilhou, 1886; Mém. Soc. Sci. Phys. et Nat. Bordeaux, 3. ser., 2 (1886), p. 201.

[f] Ztschr. Ver. Deut. Rübenz. Indus., Beilageheft, Nov., 1888.

[g] Ann. Sci. Nat. Bot., 6. ser., 9 (1878), p. 381.

[h] Ibid., 8. ser., 2 (1896), p. 275.

being certain bacteria which, without the aid of sunlight, can fashion organic cells out of mineral substances or out of very simple organic materials. We all know of the nitrous and nitric ferments which can secure the energy necessary for their life processes in purely inorganic media from the oxidation of ammonia and of nitrous acid. We know of sulphur bacteria which can build organic tissue with the aid of the potential energy in hydrogen sulphid or in elementary sulphur. We know of other organisms which derive their energy from similar oxidations of iron compounds, and we are learning to know of still other microscopic beings that make a like use of hydrogen gas and of methane for their life processes. The struggle for existence has brought forth bacteria both aerobic and anaerobic that are independent of the supply of combined nitrogen; it has evolved species which are adapted to enter into well-defined relationships with algæ or higher plants; it has produced species which develop by preference in nitrogen-rich or in nitrogen-poor media, and it has established others with specific chemical functions in the destruction or the formation of carbohydrates, of fats, of organic acids, and of lower and higher alcohols. In a word, the struggle for existence has given us innumerable bacterial species, friends and foes to plants and animals. It is among these bacterial friends that we find some of our staunchest allies in crop production. Without them and their work plants and animals could not endure on this earth, for it is reserved to them to play an essential part in the cycle of transformation to which nitrogen, carbon, hydrogen, and sulphur are subject; to play an important part likewise in the proper supply to higher plants of phosphorus, of calcium, of iron, of magnesium, and to some extent also of potassium and silicon. Every student of agriculture has come to recognize the significant rôle that must be accorded to micro-organic life in the soil in the nutrition of higher plants, yet we are far, quite far, from understanding clearly the relationships of species in the soil. The work is nearly all before us of finding a reply to an endless number of questions; of correlating the physical, chemical, and bacteriological factors in soil fertility; of deducing general principles which would serve as a basis for specific directions in specific cases. Soil-bacteriological research offers splendid opportunities for the collection of facts of utmost moment, not alone to the theory of agricultural science but also to its practice. It is safe to assert that systematic investigation in this field will reward us richly in a broader knowledge of plant-food production and plant-food assimilation. It will enable us to gauge with far greater certainty the various phases of soil fertility and to make better provision for the economic utilization of the plant food derived from soil sources or from the manures and fertilizers applied.

METHODS.

As we glance through the literature of research in soil bacteriology we note that scarcely anything was published on the subject before the beginning of the eighties of the last century. It was the introduction of Koch's[a] gelatin-plate method for the isolation of pure cultures that gave a powerful stimulus to bacteriological investigation. Koch was also among the first[b] to make quantitative soil bacteriological studies and to show that the upper layers of the soil, whether taken from densely populated districts or from fields far removed from the city, contain enormous numbers of bacteria. The work of other investigators[c] has confirmed his results. On the whole, however, these quantitative studies have not been very fruitful in their practical application, though they have given us some interesting information as to the distribution and numbers of the bacteria in the soil, as well as their relation to changing climatic conditions. Fränkel showed, for instance, that the number of bacteria rapidly diminishes with the increasing distance from the surface until they almost entirely disappear at depths below 3 meters. Similar results were obtained by Fülles, who also noted quantitative differences in the bacterial content of forest, vineyard, meadow, and field soils and pointed out that certain species may suddenly appear in predominating numbers, and as suddenly disappear. These studies and others already referred to taught us, at least, that our arable soils are inhabited by vast numbers of microscopic beings, most of them located near the surface, changing in numbers with the changing nature of soil and season and exhibiting variable group relationships.

After these preliminary studies the agricultural bacteriologists could not but recognize that the mere counting of the soil bacteria

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[a] Mitt. K. Gsndhtsamt. [Germany], I (1881), p. 1.

[b] Ibid, pp. 34–36.

[c] See Miquel. Ann. Observ. Montsouris, 1879, pp. 513–544; 1882, pp. 406–523.
- Adametz. Untersuchungen über die niederen Pilze der Ackerkrume. Inaug. Diss., Leipzig, 1886.
- Fränkel. Ztschr. Hyg., 2 (1887), pp. 521–582.
- Beumer. Deut. Med. Wchnschr., 12 (1886), p. 464.
- Maggiora. Gior. R. Accad. Med. Torino, 3. ser., 35 (1887), p. 153.
- Reimers. Ztschr. Hyg., 7 (1889), p. 307.
- Eberbach. Über das Verhalten der Bakterien im Boden Dorpats. Inaug. Diss., Dorpat, 1890.
- Fülles. Ztschr. Hyg. u. Infektionskrank., 10 (1891), pp. 225–252.
- Stålström, Finska Mosskulturför. Årsbok, 1898, pp. 44–64.
- Thiele. Centbl. Bakt. [etc.], 2, Abt., 11 (1903), p. 251.
- Löhnis. Ibid., 14 (1905), p. 1.
- Fabricius and von Feilitzen. Ibid., 14 (1905), p. 161.
- Chester. Delaware Sta. Bul. 65.
- Mayo and Kinsley. Kansas Sta. Bul. 117.

growing on certain culture media would lead to information of a very fragmentary character and altogether insufficient to allow an understanding of the direct relation of soil flora to soil fertility. To be sure, Caron[a] was inclined to attribute to the soil bacteria an important influence in plant production because of their vast numbers,[b] yet even he attempted to differentiate from among the great mass of soil organisms those of a specific physiological function. Hiltner and Störmer,[c] who after him devoted much time to quantitative studies of soil bacteriology, set out to determine the number of bacteria and the relationships of species in the upper layers of soil, but were forced to limit their field of research to a detailed study of the species that would grow on some convenient medium. Believing that even the mere determination of the number of micro-organisms in the soil may permit important conclusions, they admit, none the less, that "it should be our endeavor to arrange our method so that it may permit a determination of numbers as well as of species." In accordance with this, they arrange the organisms growing on their gelatin in three groups, viz, the liquefying, nonliquefying, and Streptothrix species, and would justify this arrangement on physiological grounds.

We thus come to distinguish two distinct conceptions in soil bacteriological investigations, one attached to numbers, the other to physiological activities. Hiltner and Störmer, strong advocates as they are of quantitative research, would yet direct this research in establishing the correlation of the various groups of soil bacteria as based on physiological characteristics. Not satisfied with knowing that such groups, for instance, as the nitrifying or nitrogen-fixing bacteria have their specific physiological functions to perform in the economy of plant nutrition, they would determine their numerical relation to one another or to other groups. There would be great promise in such work if we could assume that a given number of bacteria from any group would under identical conditions yield the same qualitative and quantitative returns as an equal number of other bacteria from the same group. To give a concrete example, we would have to assume that, everything else being equal, one nitrobacter cell can oxidize exactly as much nitrite as another nitrobacter cell. It has been pointed out by Remy[d] that the number of colonies of aerobic soil bacteria appearing on the plates shows no direct relation to the ammonifying, nitrifying, or denitrifying power of the corresponding soil,[e] an observation which forces him to the conclusion

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[a] Landw. Vers. Stat., 45 (1895), p. 401.

[b] Ibid., p. 402.

[c] Studien über die Bakterienflora des Ackerbodens, etc. Berlin, 1903, p. 445. Reprinted from Arb. K. Gsndhtsamt., Biol. Abt., 3 (1903).

[d] Centbl. Bakt. [etc.], 2. Abt., 8 (1902), pp. 657, 699, 728, 761.

[e] Ibid., p. 733.

that determining the number of the bacteria in any soil has but a very limited diagnostic value in ascertaining its fertility. Löhnis,[a] who is even more emphatic than Remy in designating mere quantitative methods as untrustworthy, says that it is quite possible that the two millions of very efficient ammonia-producing bacteria present in 1 gram of soil will accomplish more work than the five millions of bacteria present in 1 gram of another soil, when the latter, while belonging to the same physiological group, show but a relatively slight efficiency.

The same fact is also recognized by Chester[b] when he says that a soil may be low in numbers of bacteria, but contain such a bacterial flora, or combination of bacterial species which are known to be favorable to the rapid digestion of plant food, as to give it what might be termed a high bacterial potential.

Hence we find that the two methods proposed for determining the relation between soil bacteria and soil fertility are based on one or both of the conceptions of numbers and of physiological activity. The first of these proposed by Remy[c] is, briefly stated, as follows: An attempt is made to determine the physiological functions of a soil by placing weighed amounts of it (usually 10 per cent) into sterile solutions of known composition. By thus inoculating a 1 per cent peptone solution, and distilling off the ammonia formed in a given length of time, we are enabled to judge of the ammonifying power of that soil. Similarly the inoculation of solutions favorable for the development of nitrifying bacteria and the determination of the nitrates formed, the inoculation of solutions favorable for the development of denitrifying bacteria and the determination of the amount of nitrate destroyed, and the inoculation of solutions favorable for the development of nitrogen-fixing bacteria and the determination of the nitrogen fixed, offer a measure of the nitrifying, denitrifying, and nitrogen-fixing power of that soil. The other method proposed by Hiltner and Störmer,[d] like that of Remy, makes use of solutions prepared to favor in each case the development of one of the various groups of bacteria noted above, but instead of inoculation being made with 10 grams of soil per 100 cubic centimeters of nutrient solution constantly decreasing quantities are used, usually 1,000, 100, 10, 1, 0.1, 0.01, and 0.001 milligrams of soil. By this method of dilution a point is finally reached where the small quantity of soil employed contains none of the specific organisms, and hence fails to give rise to

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[a] Centbl. Bakt. [etc.], 2. Abt., 12 (1904), p. 264.

[b] Delaware Sta. Bul. 65, p. 69.

[c] Centbl. Bakt. [etc.], 2. Abt., 8 (1902), pp. 657, 699, 728, 761.

[d] Studien über die Bakterienflora des Ackerbodens, etc. Berlin, 1903, p. 474. Reprinted from Arb. K. Gsndhtsamt., Biol. Abt., 3 (1903).

the specific physiological reaction. If, for instance, no nitrifying bacteria are present in the minute quantity of soil used, no nitrification is produced in the suitably prepared ammonium-sulphate solution. By thus inoculating parallel solutions a fairly accurate estimation may be made of the number of the different bacteria present in the soil.

There is something to be said in favor of each of these methods. For one thing, Hiltner and Störmer's method is far more exact in its quantitative results than the gelatin-plate method, as was clearly demonstrated by Löhnis.[a] He found in 1 gram of soil 1,270,000 bacteria capable of developing on soil extract gelatin plates, but by using Hiltner and Störmer's dilution method he found in 1 gram of the same soil 3,750,000 peptone decomposing bacteria, 50,000 urea decomposing bacteria, 50,000 denitrifying bacteria, 7,500 nitrifying bacteria, and 25 nitrogen-fixing (in mannite solution) bacteria. Other determinations yielded confirmatory results. The great discrepancies in these two methods are largely due to the fact that many of the peptone-decomposing bacteria fail to grow on the gelatin plates, either on account of overcrowding, or of the sudden change of conditions (dilution with water and transfer to the gelatin plates), and the resulting osmotic disturbances. The discrepancies are still further intensified by the fact that such organisms as the nitrifying bacteria do not grow at all on gelatin plates, and colonies of certain aerobic nitrogen-fixing bacteria (Azotobacter) appear but seldom in mixed cultures. Moreover, the obligate anaerobes present in some soils in vast numbers form no colonies on gelatin plates under ordinary conditions of aerobic culture. And thus, while there is scarcely a doubt as to the value of Hiltner and Störmer's method for quantitative studies, it is not adapted to give us proper information as to the physiological efficiency, or, to use a term objected to in some quarters, the virulence of the several groups in any particular soil. Since, however, the amount of bacteriological change in the soil is dependent on the physiological efficiency as much as on the numbers of its bacteria, Hiltner and Störmer's dilution method is manifestly inadequate in itself to give us an exact knowledge of fertility conditions as influenced by bacteriological activities.

On the other hand, Remy's method has been criticised by Hiltner and Störmer[b] on account of the proportionately large quantities of soil used for inoculation. It is claimed by them that the great number of organisms thus introduced into the nutrient solution may contain species that would multiply very rapidly and suppress the activities of those bacteria whose physiological efficiency it was

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[a] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), pp. 2, 3.

[b] See Löhnis. Centbl. Bakt. [etc.], 2. Abt., 12 (1904), p. 264.

intended to study. This may be illustrated by the changes in a nitrate solution of certain composition made up to favor the growth of denitrifying bacteria. The 10 grams of soil introduced into 100 cubic centimeters of this solution might cause in a given length of time the entire disappearance of the nitrate without a diminution in the amount of total nitrogen, and if we depended on qualitative tests alone we might be led to assume that the nitrate had been destroyed with the evolution of gaseous nitrogen. A quantitative determination would soon convince us, however, that there was here merely a transformation of the nitrate nitrogen into organic nitrogen and no denitrification proper. The extent of this possible transformation and the nature of the organisms involved are discussed by Gerlach and Vogel[a] and more recently by Löhnis.[b]

After due allowance is made for this objection to Remy's method, it still remains true that there is promise in it of future usefulness as an aid in the study of soil fertility problems. It does not seem practicable to meet this objection by using smaller quantities of soil, for Löhnis could not secure satisfactory agreement in the duplicates where only one-half gram to 2 grams of soil was used for inoculation. His critical comparison of the two methods convinced him that trustworthy results in investigations on the efficiency of the various groups of soil bacteria may be secured only when relatively abundant amounts of soil are used for the inoculation of suitable solutions.[c] In order, however, to discover finer differences in soils of the same origin, he found himself obliged to modify Remy's method, in that he used soil extract with the addition of suitable reagents instead of the artificial nutrient solutions employed by Remy. He thus secured differences in denitrification and nitrogen fixation where the unmodified method failed to disclose any appreciable differences. Ehrenberg[d] and Wohltmann, Fischer, and Schneider[e] also examined Remy's method in its application to soil fertility studies. The former was led to conclude that improved bacteriological methods of soil examination deserve consideration together with or after vegetation experiments and chemical-physical analysis,[f] while the latter investigators were able to trace a distinct connection, in some cases, between the bacterial activities in the soil and its previous chemical treatment.[g]

[a] Centbl. Bakt. [etc.], 2. Abt., 7 (1901), p. 609.
[b] Ibid., 14 (1905), p. 598.
[c] Ibid., 12 (1904), p. 460.
[d] Landw. Jahrb., 33 (1904), p. 1.
[e] Jour. Landw., 52 (1904), p. 97.
[f] Landw. Jahrb., 33 (1904), p. 131.
[g] Jour. Landw., 52 (1904), p. 124.

Löhnis sums the matter up by saying:[a]

> In order to secure results which would permit us to determine how the cultivation and fertilization of the soil, as well as the nature of the crop, weather, and season have exerted an influence on the bacteria important to agriculture, and likewise on the transformations caused by them, we are constrained for the present to follow the methods that were first logically examined by Remy, involving the determination of the effect produced by a certain quantity of soil in properly constituted media. To be sure, only approximate results are obtained even here, yet the nature of these is such as to allow a ready control by soil and field experiments. It may be claimed with much justice that, fundamentally, this would be a chemical proceeding, yet there seems to me a great advantage in this very thing. The bacteriological basis having been clearly established by the investigations of the agricultural bacteriologist, further valuable research in this direction could be carried out in agricultural chemical laboratories by persons properly qualified for such work. Such amplification would not be to the disadvantage of chemical soil analysis, while the soil bacteriological investigators could devote themselves to fruitful special research, instead of rather worthless and spirit-destroying countings.

More recent studies by Lipman,[b] Löhnis,[c] Buhlert and Fickendey,[d] and Gutzeit[e] have served as a further test of Remy's chemical bacteriological methods for the study of soil fertility. Lipman proposed the use of soil infusions instead of soil in the study of ammonification and showed that by shaking a weighed quantity of soil in sterile water for five minutes practically all of the bacteria are brought into suspension. Quantities of this suspension equivalent to 10 per cent of soil when pipetted into sterile peptone solutions to which potassium phosphate and magnesium sulphate had been added evolved as much ammonia in a stated time as did the soil itself.[f] It was found that the coarser-grained, sandy soils allowed all of their bacteria to be brought into suspension by one or two minutes shaking, while the fine-grained clay soils still showed differences between the ammonifying power of the three-minute suspension and the five-minute suspension. Longer shaking than five minutes seemed not to increase the amounts of ammonia liberated by the bacteria in suspension. It was demonstrated, likewise, that in the case of ammonification, at least, there are two distinct factors which determine the extent of the bacterial changes, namely, the bacteria themselves and the chemical constitution of the soil used. Sterile portions of one and the same soil inoculated with infusions from different soils yielded varying amounts of ammonia, as did also sterile portions of different soils inoculated with one and the same infusion. It was

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[a] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 7; 17 (1906), p. 518.
[b] New Jersey Stas. Rpts. 1905, p. 225; 1906, p. 117.
[c] Centbl. Bakt. [etc.], 2. Abt., 15 (1905), p. 361.
[d] Ibid., 16 (1906), p. 399.
[e] Ibid., p. 358.
[f] Data still unpublished.

shown, thus, that bacterial changes in the soil as measured by their chemical products are a function of the numbers and physiological efficiency of the bacteria on the one hand and of the chemical composition of the soil on the other. This fact is of great significance, not only for soil-inoculation problems, but for all soil-improvement problems. It shows that the bacterial flora of any soil bears an intimate relation to its composition and that an enhancement of the desirable bacterial activities in the soil can only be encouraged by the proper improvement in the physical and chemical composition of the soil. Furthermore, a number of cylinder soils which had been differentiated in the course of eight years by varying nitrogen treatment showed marked differences in their ammonifying power, in agreement with similar differences that became apparent in the crop yields.

Differences of a like nature were brought out by the recent investigations of Löhnis.[a] He found that it is possible, by the inoculation of suitable solutions with 10 per cent of soil to secure valuable information concerning the transformations in the soil induced by micro-organisms. In his application of this method each of the transformations tested indicated a distinct reaction affected by temperature conditions, precipitation, and mechanical treatment of the soil, and this reaction was more or less prominent, according to the physiological peculiarities of the several groups of bacteria involved. The results of these transformation experiments, in so far as they could be tested by field trials and bacteriological examinations, were found to agree with the latter.

It may be assumed that more extensive investigations in this direction will permit in time the discovery, on the one hand, of the general laws controlling the main factors in the direction and intensity of the agriculturally important transformations in the soil and a clear understanding, on the other hand, of the variations in different soils as dependent on bacteriological factors.

The extract prepared from the soils to be examined was found to be peculiarly useful as a basis for the preparation of the different solutions in Löhnis's investigations, and he is of the opinion that further tests in this direction are desirable, since in the comparison of different soils allowance is thus made to a greater or less extent for the chemical differences in the soils.

Buhlert and Fickendey[b] have also used the soil-extract media employed by Löhnis. For inoculation they used soil infusion instead of soil. With this modification of Remy's method they compared aerated and nonaerated soil of the same origin, and found that the former split off less ammonia and produced less nitrate than did the

[a] Centbl. Bakt. [etc.], 2. Abt., 15 (1905), p. 434. [b] Ibid., 16 (1906), p. 399.

corresponding nonaerated soil. On the other hand, the aerated soil showed a greater power of denitrification and of nitrogen fixation than did the corresponding nonaerated soil.

Still more interesting in this connection is the work of Gutzeit[a] on nitrogen transformation in the soil as affected by the character of the plants growing upon it. It had long been suspected that reciprocal relations between various groups of soil bacteria and the crops growing upon the soil actually exist, yet we lacked the facts to justify this supposition. These facts were furnished by the chemical-bacteriological methods discussed above, and Gutzeit found that they are splendidly adapted for the solution of agricultural-bacteriological problems. He demonstrated that plats seeded to oats showed marked differences in the nitrifying power of the different soils as affected by the growth among the oat plants of a large number of wild mustard plants, and what is even more significant, he found these differences to persist in a less pronounced degree in the following season. He concludes that the injury to cultivated plants caused by weeds, like the wild mustard, is due not alone to the unfavorable effect on the general conditions of growth and nutrition, but also under certain circumstances to influences on the bacterial life in the soil in a direction unfavorable to the cultivated crop, as, for instance, the interference with nitrification by the removal of larger quantities of lime and of moisture, an interference which may show its effect over a considerable length of time. Hence he adds that the bacteriological-chemical methods—that is, the quantitative determination of transformation products in nutrient media inoculated with larger quantities of soil—possess a reliability and exactness that make them suitable, apparently, for the solution of agricultural-bacteriological problems. This statement is well borne out by the data recently secured in the laboratories of the New Jersey Experiment Station. These data, still unpublished, justify the claim made by Löhnis and Parr[b] that there is no general "decay power" characteristic of any one soil, since varying conditions of season, temperature, and moisture affect the decomposition of different organic manures.

Our knowledge of soil-bacteriological processes has gained much from the investigations of the effect of carbon bisulphid on soil fertility. It was observed in 1894, by Oberlin[c] in Germany, and Girard[d] in France, that the application of carbon bisulphid increased the crop-producing power of the soil. Oberlin showed, moreover, that grape-

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[a] Centbl. Bakt. [etc.], 2. Abt., 16 (1906), p. 358.

[b] Ibid., 17 (1906), p. 518.

[c] Bodenmüdigkeit und Schwefelkohlenstoff. Mainz, 1894. Jour. Agr. Prat., 59 (1895), I, pp. 459, 499, 535.

[d] Compt. Rend. Acad. Sci. [Paris], 118 (1894), p. 1078.

sick soils could be rejuvenated by the use of carbon bisulphid, and founded on this his system of grape culture, where fallowing and rotation could be dispensed with in the resetting of vineyards. The striking influence of carbon bisulphid on soil fertility created much discussion, particularly as to the manner of its action. The existing uncertainty of opinion was voiced by Wollny when he said in 1898 that no explanation has been found as yet to account for the beneficial influence of carbon bisulphid on the crop-producing power of cultivated soils. Koch,[a] who has devoted much time to the question, is inclined to ascribe the favorable effect of carbon bisulphid to its stimulating action on plant growth, but he has found no support for his opinion. Most of the investigators who have examined this problem, and particularly Hiltner and Störmer,[b] and Krüger and Heinze[c], ascribe the action of carbon bisulphid to its influence on the bacteria in the soil. Indeed, the evidence supplied by these investigators is quite convincing, and it sheds not a little light on the general question of soil fertility as affected by micro-organic life. Hiltner and Störmer found that under normal conditions there is a certain equilibrium established among the various groups of soil bacteria, and that the organisms capable of growing on meat-extract gelatin are composed of Streptothrix species 20 per cent, gelatin-liquefying species 75 per cent, and nonliquefying species 5 per cent. But when carbon bisulphid is applied to the soil, its bacterial inhabitants are injured, though not completely destroyed, the injury varying with the changing conditions of temperature, moisture, and amount of carbon bisulphid applied, as well as the duration of its action. Not all of the bacterial species are depressed in their development to an equal extent, the injury being most pronounced in the Streptothrix species, and least pronounced in the gelatin-liquefying species. The depressing action of carbon bisulphid disappears after a shorter or longer interval, and is followed by a very rapid multiplication of the micro-organisms in the soil. The equilibrium having been destroyed, however, the new development follows along different channels, and there occurs not only an enormous increase in the total number of soil bacteria, but also an abnormal predominance of certain species. The new conditions thus established for a time favor a more ready utilization of the stores of soil nitrogen, and likewise the fixation of atmospheric nitrogen by certain bacterial species. It is for this reason that the application of carbon bisulphid is followed after a time by a very decided increase in crop yields as compared with the corresponding yields from soils not treated. Increased harvests under such conditions have been

[a] Arb. Deut. Landw. Gesell., 1899, No. 40.

[b] Studien über die Bakterienflora des Ackerbodens, etc. Berlin, 1903, p. 477. Reprinted from Arb. K. Gsndhtsamt., Biol. Abt., 3 (1903).

[c] Centbl. Bakt. [etc.], 2. Abt., 16 (1906), p. 329.

observed by Oberlin, Krüger and Heinze, Koch, Moritz and Scherpe, Caron, Behrens, Girard, and others.[a] Wollny comes to the following conclusion in regard to the action of carbon bisulphid:

(1) The application of carbon bisulphid to the soil within the growing season may lead, according to the amount introduced, to a complete destruction of the growing crop, or to a temporary retardation merely, involving a greater or slighter depression in the production of plant substance.

(2) The application of carbon bisulphid several months before planting increases to a very considerable extent the fertility of the soil. This influence is felt, according to the amount of carbon bisulphid used, through one or several growing seasons, after which, when no manure or fertilizer had been applied meanwhile, a marked decrease in the yields becomes evident.

(3) The bacteria concerned in the decomposition of organic substances and the formation of nitrates in the soil, as well as the tubercle bacteria of the legumes are not destroyed, even by the application of large quantities of carbon bisulphid, but are only hindered for a time in their development.

Hiltner and Störmer, who studied the question in greater detail, and at a later date, conclude that—

(1) By destroying the existing bacterial equilibrium in the soil, the carbon bisulphid opens the way for an entirely new bacterial development. This is achieved through the unequal retardation in the growth of the different groups of bacteria. Hence certain groups become disproportionately prominent, while others are almost entirely suppressed.

(2) The rapid increase in the numbers of the bacteria is followed by a more intense transformation of plant-food substances. Decomposition and fixation processes result in an accumulation of readily available nitrogen compounds utilized by the crops. Hence the action of carbon bisulphid is in the nature of nitrogen action.

(3) The initial suppression of the nitrifying species becomes of advantage in that the nitrogen compounds, simplified by other species, are prevented from being rapidly changed into nitrates and being leached out of the soil.

(4) The more or less permanent suppression of the denitrifying organisms must be regarded as an additional factor favoring plant growth.

The introduction of the poison into the soil decimates at first its bacterial flora, but with the disappearance of the injurious carbon-bisulphid vapors it also encourages a vigorous and long-continued increase of the organisms, resulting in an increase of the store of more readily available nitrogen. It is still to be determined whether this increase is largely due to the fixation of atmospheric nitrogen or to the unlocking of the vast store of combined nitrogen in the soil. It is most probable, however, that even though one of these processes predominate, the other is surely more extensive than it would be in normal soil. The nitrogen thus secured is not at once made accessible to the higher plants, but is at first laid fast in the bacterial bodies. This assumption would best explain the fact that plants growing upon a soil treated with carbon bisulphid show retarded growth, even some time after the application of the latter,

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[a] See Behrens. Jahresber. Gärungs-Organismen, 6 (1895), p. 280.
Chandon de Briailles. Rev. Vit., 4 (1895), p. 320.
Gerlach. Jahresber. Landw. Vers. Stat. Jersitz-Posen, 1896, p. 3; abs. in Centbl. Agr. Chem., 27 (1898), p. 716.
Morgen. Ber. Thät. Landw. Chem. Vers. Stat. Hohenheim, 1896, p. 73; 1898, p. 46.
Pagnoul. Ann. Agron., 21 (1895), p. 222.
Wagner. Deut. Landw. Presse, 22 (1895), No. 14, p. 123.

and the explanation hitherto accepted that the injury results from the direct action of the poison seems hardly reasonable after our discovery that the most intense bacterial activities are asserting themselves just at that time. The nitrogen fixed in the bacterial bodies is gradually rendered soluble by decomposition processes, and thereby made accessible to nitrification and the higher plants. Hence when the carbon bisulphid is applied in the fall, there is enough time left until the planting of the following spring crop for the mineralization of the bacterial nitrogen. It is quite evident, of course, that the nitrogen combined in the bodies of generations of bacteria is not all made soluble within a single year, but only in the course of several growing seasons, so that we may readily account for the increased harvests secured for two or more successive years after strong applications of carbon bisulphid, even though the bacterial transformations had by that time declined. The exhaustion of the soil finally manifesting itself after a shorter or longer time may be explained by the deep-seated changes in the bacterial soil flora, which does not return so easily to its normal state. It is quite possible that the return to the normal conditions is prevented by the exhaustion for years to come of the more available portions of the plant nutrients.

Heinze[a] agrees with Hiltner and Störmer that the influence of carbon bisulphid is much like that of a nitrogenous manure. He says:

The action of carbon bisulphid undoubtedly exhibits the same characteristics as that of nitrogenous substances, as is clearly evidenced by the dark green color and the vigorous development of the plants. Subsequently there may be observed a decided tendency of grain crops to lodging, just as if too great quantities of nitrogen were at their disposal. On the whole, we must seek the main course of the beneficial effect of carbon bisulphid on the soil in the enormous increase of soil organisms at the proper time. Various data could be furnished to convince even the most skeptical that there is justification for the assumption that the vast increase in the numbers of the various soil organisms must be followed by a great increase in the store of nitrogen available to plants.

It was further shown by the studies of Krüger and Heinze that the large amounts of nitrogen thus made available to the crops are derived partly from soil sources and partly from atmospheric sources. They not only demonstrated that soils treated with carbon bisulphid showed an increase in their total nitrogen content, but also that this increase was the result of the more vigorous growth of the nitrogen-fixing Azotobacter species. Heinze states, therefore, that the significance of carbon bisulphid for the entire nitrogen question may be summarized as follows:

The initial suppression of amid-ammonia formation and of nitrification creates favorable conditions for the development of nitrogen-fixing bacteria, while the subsequent more intense transformation of the bacterial proteids and of other nitrogenous organic substances into amido and ammonium compounds and the following vigorous nitrification processes place at the disposal of the plant an abundant and uniform supply of soluble nitrogen compounds. The various organic materials in the soil—such as plant residues, pectins, pentosans, humic substances, etc.—may furnish the carbon food for the Azotobacter species, and suitable carbon compounds may also be furnished by algæ and molds.

The action of carbon bisulphid as thus examined in detail may help us to understand the peculiar effects at times produced by the turning

[a] Centbl. Bakt. [etc.], 2. Abt., 16 (1906), p. 329.

under of mustard, buckwheat, rye, and of other nonleguminous crops. It has been noted repeatedly that these crops when plowed under in a green state led to a better growth of the following cereal or root crops on nitrogen-poor soils. So striking were the benefits in some instances from green manuring with mustard that it was proposed in some quarters to include mustard among the "nitrogen gatherers" rather than among the "nitrogen consumers." The first communication of Hellriegel and Wilfarth on the nitrogen-fixation by legumes, made twenty years ago, and the stimulated research in this field that followed their work, soon furnished conclusive proof that mustard is incapable of utilizing atmospheric nitrogen for its growth. But, as Heinze points out, there may have been more or less justification for this belief, so far as the indirect influence of mustard is concerned. It would seem that at times the action of mustard is not unlike that of carbon bisulphid in affecting the bacterial flora of the soil, and it really appears from facts already known that the green mustard substance in the soil retards the development of the acid-forming species and encourages the growth of the nitrogen-fixing Azotobacter species. Heinze thinks, therefore, that further study may enable us to make extensive use of mustard as an indirect source of combined nitrogen, and tries to find theoretical support for his belief in the fact that allyl mustard oil, $C_3H_5—N=C=S$, a constituent of the mustard plant, may be regarded as a derivative of carbon bisulphid.

BACTERIAL DECOMPOSITION OF NONNITROGENOUS ORGANIC SUBSTANCE.

We have considered thus far the decomposition of organic materials in the soil from the nitrogen standpoint largely. It would be well to remember at the same time that there are numerous bacterial reactions in the soil, which primarily concern nonnitrogenous compounds. Specific organisms have been isolated, which can utilize for their development formates, acetates, propionates, butyrates, valerates, and the corresponding salts of the higher fatty acids, or such carbohydrates and allied compounds as arabinose, levulose, dextrose, galactose, maltose, saccharose, and dextrin; or alcohols, like ethyl alcohol, propyl alcohol, and butyl alcohol; or glycerin, mannite, sorbite, erythrite, and dulcite. Among these more or less specific reactions there are some which deserve more than a passing mention. This is particularly true of the fermentation of cellulose, a process of extreme importance to plant and animal life. Pure celluloses are well defined chemical compounds, having the general empirical formula $C_NH_{2M}O_M$, are insoluble in ordinary solvents and resistent to hydrolyzation. The term cellulose is frequently and quite incorrectly used to designate the mixture of compounds, of which the cell walls of plants are com-

posed. The bacterial decomposition of cellulose as a specific process was investigated quite thoroughly by Omelianski,[a] to whom we should be grateful for much definite information on the subject. It was suspected by Mitscherlich, as far back as 1850, that the destruction of the cell walls of potatoes noted by him was due to micro-organisms. Trécul, who examined in 1865 the bacteria occurring in macerated plant tissues, observed some that were colored blue by iodin, and named them Amylobacter. Van Tieghem, who studied impure cultures of these organisms between 1877 and 1879, ascribed to them the power of decomposing cellulose, but Omelianski is inclined to think that Van Tieghem's Amylobacter consisted largely of impure cultures of pectin ferments,[b] rather than of cellulose ferments. The studies of Popoff, Tappeiner, and Hoppe-Seyler dealt with the decomposition of pure cellulose in filter paper by impure cultures of cellulose ferments. They all observed the evolution of methane, of carbon dioxid, and in some cases, also, of hydrogen. Dealing with impure cultures, their experiments were not calculated to furnish exact information as to the nature of the agent or agents causing the destruction of cellulose. The studies of Omelianski, begun in the spring of 1894 and reported in 1895, 1897, 1902, 1904, and 1906, have demonstrated that there are two distinct species of anaerobic ferments capable of destroying cellulose—one of them a hydrogen ferment, the other a methane ferment. An examination of the gases evolved showed in the one instance a

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[a] Centbl. Bakt. [etc.], 2. Abt., 8 (1902), p. 193; 11 (1904), p. 369; 12 (1904), p. 33; 15 (1906), p. 673.

[b] See Behrens. Centbl. Bakt. [etc.], 2. Abt., 8 (1902), p. 114.
Hauman. Ann. Inst. Pasteur, 16 (1902), p. 379.
Störmer. Centbl. Bakt. [etc.], 2. Abt., 13 (1904), p. 34.
Mitscherlich. Ber. Bekanntmach. Geeigneten Verhandl. K. Akad. Wiss. Berlin, 1850, p. 102.
Popoff. Arch. Physiol. [Pflüger], 10 (1875), p. 113.
Van Tieghem. Compt. Rend. Acad. Sci. [Paris], 88 (1879), p. 205; 89 (1879), p. 5.
Tappeiner. Ber. Deut. Chem. Gesell., 15 (1882), p. 999; 16 (1883), pp. 1734, 1740; Ztschr. Biol., 19 (1883), p. 228; 20 (1884), p. 52.
Hoppe-Seyler. Ztschr. Physiol. Chem., 10 (1886), pp. 201, 401; 11 (1887), p. 561.
Trécul. Compt. Rend. Acad. Sci. [Paris], 61 (1865), p. 156; 65 (1867), p. 513.
Gayon. Compt. Rend. Acad. Sci. [Paris], 98 (1884), p. 528.
Schloesing. Compt. Rend. Acad. Sci. [Paris], 109 (1889), p. 835.
Van Senus. Bijdrage tot de Kennis der Cellulose-gisting. [Proefschrift.] Leyden, 1890.
Omelianski. Compt. Rend. Acad. Sci. [Paris], 121 (1895), p. 653; 125 (1897), pp. 970, 1131; Arch. Sci. Biol. [St. Petersb.], 7 (1899), p. 411.
Scheunert. Ztschr. Physiol. Chem., 48 (1906), p. 25.
Oppenheimer. Ztschr. Physiol. Chem., 48 (1906), p. 240.
Ruge. Sitzber. K. Akad. Wiss. Wien, 2. Abt., 44 (1861), p. 739.
Kerry. Monatsh. Chem., 10 (1889), p. 864.
Zoja. Ztschr. Physiol. Chem., 23 (1897), p. 236.

maximum content of hydrogen of 85.85 per cent, in the other instance a maximum content of methane of 75.7 per cent. Acetic and butyric acids, smaller quantities of valerianic acid, and probably traces of formic acid were also produced. Omelianski summarizes his results by saying that cellulose may undergo either a hydrogen or methane fermentation; that one is independent of the other and is due in each case to a specific organism; that the two organisms are morphologically much alike, the comparison showing the methane bacillus to be somewhat the smaller of the two; that their physiological characteristics are also much alike, the two requiring similar conditions for their development, and forming similar products. The single characteristic by which they may be easily distinguished is the production of hydrogen by the one and the production of methane by the other. Omelianski regards these two organisms as the cellulose ferments par excellence, and shows that quantitatively their work even in pure culture is quite extensive, and compares favorably with the production of methane in manure heaps, as determined by Gayon and Schloesing. There is nothing to prevent us, therefore, says Omelianski, from ascribing to the two organisms a prominent rôle in the natural processes of cellulose decomposition. Even if there be discovered in the future, a whole series of cellulose ferments, there will still remain for the organisms described by us the most difficult task of the decomposition of the cellulose forming the residual product of preceding reactions, and at a time when the protein bodies and soluble carbohydrates had already been destroyed, and the mineralization of the substratum, exclusive of the residual cellulose, had been carried to completion.

In well-ventilated soils the production of methane may be reduced to a minimum, or even entirely eliminated. Nevertheless, the formation of methane in nature is quite extensive, this gas issuing from volcanoes, from wells in the oil regions, and appearing in coal-bearing strata. Its origin under such conditions is probably due to inorganic reactions ($CaCO_3 + SO_2 + 3H_2S = CaSO_4.H_2O + 3S + CH_4$) (Roche). On the other hand, the well-known formation of methane in moist soils, meadows, lakes, ponds, and swamps is evidently of bacterial origin. Reference has already been made to the formation of methane in manure heaps and Tappeiner pointed out that large quantities of this gas are formed in the digestive tract of herbivorous animals. He says that the exact mechanism of methane formation as due to bacterial activities has not been made clear as yet. Omelianski suggests the general reaction $2C_6H_{10}O_5 = 5CO_2 + 5CH_4 + 2C$. He says:

It is possible that a reaction of this sort forms the basis of the universal processes of humification—that is, the gradual transformation of organic substances into a mixture of brown and black substances with a high carbon content, such as is characteristic of fossil coals. But whatever the mechanism of these transformations, the active participation of micro-organisms in the latter can not be denied.

Omelianski points out, moreover, that methane may be produced not only from cellulose and acetates, but also from pentoses, pentosans, butyrates, lactates, and protein bodies, and claims that the number of the various reactions in nature which involve the formation of methane is no smaller, perhaps, than that of the fermentation processes leading to the evolution of hydrogen.

The enormous amounts of methane and of hydrogen that escape into the atmosphere in the manner just indicated do not apparently accumulate there, for repeated analytical studies have failed to show any but the merest traces of these gases in atmospheric air. In view of the fact that both hydrogen and methane, in the quantities produced, represent a large amount of potential energy, it becomes quite interesting to inquire as to the mode of their disappearance. It was noted by Immendorff as early as 1892[a] that hydrogen and oxygen may be made to unite under the influence of soil. He observed that such oxidation of hydrogen was brought about only by normal soil, but not by soil that had been previously subjected to the action of chloroform vapors. This interesting observation apparently passed unnoticed for more than a decade. Two papers recently published, one by Kaserer[b] and the other by Söhngen,[c] serve to throw a new light on this phase in the cycle of carbon and hydrogen transformation. An inorganic solution containing dipotassium phosphate, ammonium chlorid, magnesium sulphate, sodium bicarbonate, and a trace of ferric chlorid, and confined in an atmosphere consisting of a mixture of hydrogen, oxygen, and carbon dioxid, was inoculated by the former with a small quantity of soil. Growth took place and the hydrogen disappeared. The presence of a small quantity of carbon dioxid seems necessary for the development of the organisms, and it would appear that like the nitrifying bacteria they can produce bacterial protein in inorganic solutions, deriving their carbon from carbon dioxid. This reaction is of great significance in agriculture, for a great loss of energy is prevented by the bacterial oxidation of hydrogen formed in the deeper layers of the soil by anaerobic ferments. This reaction also partly counteracts the rapid mineralization of organic materials, in that it leads to the formation of complex compounds out of hydrogen, oxygen, and carbon dioxid. It is, of course, still to be determined what the quantitative significance of these processes may be; meanwhile we find in them an interesting topic for speculation as to the function of certain bacteria in the energy relations of the earth.

Other organisms capable of utilizing methane as the sole source of energy in their life-processes were found by both of the investigators named above. Söhngen secured pure cultures of an organism which

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[a] Landw. Jahrb., 21 (1892), p. 281.

[b] Centbl. Bakt. [etc.], 2. Abt., 15 (1905), p. 573.

[c] Ibid., p. 513.

he named *Bacillus methanicus.* When grown in inorganic solutions, confined in an atmosphere of one-third methane and two-thirds air, it caused the disappearance of the former, with the production of considerable quantities of organic matter. These studies are the more interesting since they add to the list of soil bacteria organisms hitherto unknown and capable, like the nitrifying bacteria of developing in inorganic solutions, and of fashioning in the dark protein substances out of carbon dioxid or methane in the presence of the simple salts in the nutrient solution.

The bacterial reactions just noted may serve likewise as a link in the chain of evidence that is being gathered by the plant physiologist in his attempts to explain the nature of carbon assimilation by green plants. It is agreed that this process calls for at least two conditions: (1) The supply of energy from without; and (2) the presence of chlorophyl in the leaves. The reaction is essentially endothermal, with sunlight serving as the source of energy. It is not known how the chlorophyl makes possible the assimilation of carbon. The claim has been made [a] and disproved that the chlorophyl itself is the first product of assimilation. The suggestion has been made,[b] also, that it is the function of the chlorophyl to withdraw oxygen from the carbon dioxid. This suggestion, while not supported by experimental proof, has much to recommend it. An hypothesis proposed by Baeyer[c] assumes that the carbon dioxid is reduced to formaldehyde and that the latter is transformed into sugar by condensation. This hypothesis finds substantial proof in the investigations of Löb,[d] for the latter succeeded in demonstrating that, with energy supplied from the outside, moist carbon dioxid may give rise to formaldehyde. Preceding studies by a number of investigators had established the fact that silent electrical discharges at ordinary temperatures may lead to the decomposition of carbon dioxid into carbon monoxid and oxygen, and likewise that the carbon monoxid thus formed is capable of serving as the starting point for further synthetical changes. Löb's extended researches with specially constructed apparatus seem to indicate that there is an analogy between the reactions thus induced and those occurring in the synthesis of carbohydrates by green plants. Carbon dioxid in the presence of water, when acted upon by silent electrical discharges, gave him the following reactions:

(1) $2CO_2=2CO+O_2$
(2) $CO+H_2O=HCOOH$
(3) $CO+H_2O=CO_2+H_2$
(4) $3O_2=2O_3$
(5) $2H_2+2O_3=2H_2O_2+O_2$
(6) $H_2+CO=H_2CO$

[a] Sachsse. Chem. Centbl., 3. ser., 12 (1881), pp. 169, 185, 236.

[b] Pfeffer. Pflanzen-Physiologie. Leipzig: Wilhelm Engelmann, 1897, vol. 1, 2. ed., p. 268.

[c] Ber. Deut. Chem. Gesell., 3 (1870), p. 63.

[d] Landw. Jahrb., 35 (1906), No. 4, p. 541.

The products secured by him were: Carbon monoxid, formic acid, hydrogen peroxid, oxygen, and some ozone. The further important discovery was made by him that the formation of hydrogen in the reaction between carbon monoxid and water is followed by the combination of hydrogen with more carbon monoxid to produce formaldehyde. The proof thus furnished that formaldehyde (and therefore carbohydrate) appears as a direct reaction product of moist carbon dioxid offers strong support for Baeyer's hypothesis. The formation of formaldehyde with the aid of silent electrical discharges may, therefore, be divided into three phases:

$$
\begin{aligned}
&(1)\ 2CO_2=2CO+O_2\\
&(2)\ CO+H_2O=CO_2+H_2\\
&(3)\ CO+H_2=HCOH
\end{aligned}
$$

The formation of formaldehyde could best be detected where the removal of the oxygen was provided for by the addition of such substances as pyrogallol, or, better still, chlorophyl.

Carbon monoxid, water, and hydrogen, under the influence of silent discharges, produced formic acid and formaldehyde, and also another polymerization product of carbon dioxid and hydrogen, glycolaldehyde. The latter is not only the simplest sugar, but is also readily transformed into higher sugar—tetroses and hexoses. Hence,

$$
\begin{aligned}
&(1)\ CO_2+H_2O=CO+H_2+O_2\\
&(2)\ H_2+CO=H_2CO\\
&(3)\ 2(H_2+CO)=CH_2OH.CHO\\
&(4)\ 6H_2CO=C_6H_{12}O_6\\
&(5)\ 3CH_2OH.CHO=C_6H_{12}O_6
\end{aligned}
$$

Formaldehyde itself, when exposed to silent discharges, is decomposed into carbon monoxid and hydrogen; thus

$$CH_2O=CO+H_2$$

In the presence of water the reaction may be carried further with the formation of carbon dioxid and water.

$$CO+H_2O=CO_2+H_2$$

Through a more far-reaching reduction of the carbon monoxid there may be formed methane. The latter is always formed when an excess of hydrogen is brought together with carbon monoxid. Methane and carbon monoxid may in turn unite with the formation of acetaldehyde:

$$CH_4+CO=CH_3CHO$$

Acetaldehyde unites with hydrogen to form ethyl alcohol. The following reactions may, therefore, be noted here:

$$
\begin{aligned}
&(1)\ 2CO_2=2CO+O_2\\
&(2)\ 2CO+2H_2O=2CO_2+2H_2\\
&(3)\ CO+3H_2=CH_4+H_2O\\
&(4)\ CH_4+CO=CH_3CHO\\
&(5)\ CH_3CHO+H_2=CH_3CH_2OH
\end{aligned}
$$

It seems to be the function of the chlorophyl to combine with part of the oxygen of the carbon dioxid, assuring thus its continuous dissociation. Hence it would appear that the chlorophyl performs functions opposite to those of hemoglobin, since it is the office of the latter to carry atmospheric oxygen to the organism, whereas the chlorophyl carries the oxygen from the organism into the atmosphere.

Turning now to the bacteriological reactions bearing on this point, we find much interesting information in the researches of Kaserer.[a] The latter studied in this connection two organisms isolated by him, the first of them apparently a new species, which he named *Bacillus pantotrophus*, the other identical with *B. oligocarbophilus* isolated by Beijerinck and Van Delden. *B. pantotrophus* was found to possess the remarkable ability to build organic substance from hydrogen and carbon dioxid as well as from a large number of various organic compounds. This facile adaptation to environment distinguishes it sharply from other autotrophic organisms. The chemical reactions involved in the oxidation of hydrogen and the accompanying formation of bacterial substance could be expressed thus:

$$(1)\quad \underset{OH}{\overset{OH}{CO}}+2H_2=\underset{H}{\overset{H}{CO}}+2H_2O+6\text{ Cal.};\ CH_2O+O_2=H_2CO_3+132\text{ Cal.}$$

(172 Cal.) (40 Cal.) (2.69 Cal.) (40 Cal.) (172 Cal.).

$$(2)\quad \underset{OH}{\overset{OH}{CO}}+H_2=CO+2H_2O-5\text{ Cal.};\ 2\ CO+O_2+2H_2O=2H_2CO_3+148\text{ Cal.}$$

(172 Cal.) (29 Cal.) (2.69 Cal.) (2.29 Cal.) (2.69 Cal.) (2.172 Cal.).

$$(3)\quad \underset{OH}{\overset{OH}{CO}}+H_2=\underset{H}{\overset{OH}{CO}}+H_2O-1\text{ Cal.};\ 2HCOOH+O_2=2H_2CO_3+140\text{ Cal.}$$

(172 Cal.) (102 Cal.) (69 Cal.) (2.102 Cal.) (2.172 Cal.).

It was demonstrated by Kaserer that the oxidation of hydrogen by *B. pantotrophus* is carried out according to formula 1, while *B. oligocarbophilus* seems to effect it according to formula 2. He has not succeeded in finding an organism that oxidizes hydrogen according to formula 3. When a nutrient solution containing 0.05 per cent of K_2HPO_4, 0.02 per cent of $MgSO_4$, 0.10 per cent of NH_4Cl, 0.05 per cent of $NaHCO_3$, and a trace of $FeCl_3$, and confined in an atmosphere of oxygen and carbon dioxid, is inoculated with soil there takes place, at first, a development of organisms which need organic substances for their growth. With the exhaustion of the organic compounds these species are gradually suppressed, and in about five days the hydrogen begins to disappear at the rate of 0.1 to 0.2 centimeters per day in 5-centimeter fermentation tubes. The bacterial flora becomes entirely changed in its character, and the

[a] Centbl. Bakt. [etc.], 2. Abt., 16 (1906), p. 681.

new species which become prominent may or may not be accompanied in their growth by the formation of a membrane. Where no membrane is formed, the culture is found to contain a motile bacillus whose yellowish colonies grow on gelatin only after four to six days. Where a membrane is formed, the latter is found to consist of numerous very small, nonmotile bacteria, apparently identical with *B. oligocarbophilus* of Beijerinck and Van Delden. The motile bacillus referred to above was found capable, also, when growing in pure culture, of oxidizing hydrogen. Because of its ability to develop autotrophically in hydrogen, oxygen, and carbon dioxid, and also heterotrophically on most organic media, Kaserer proposed to name it *B. pantotrophus*. The organism is distinctly aerobic, and does not, therefore, grow without oxygen; nevertheless the oxygen introduced with the hydrogen and carbon dioxid is sufficient for its needs. Considerable quantities of organic matter may be accumulated by renewing the supply of hydrogen in the bell jar where the organism is growing on silica jelly. Its ability, therefore, to oxidize hydrogen when growing on silica jelly proves that it is autotrophic. It grows likewise quite well on most media, and can make use of such compounds as peptone, mannite, cane sugar, dextrose, and to a more limited extent of glycerin, urea, and formates as a source of carbon. As a source of nitrogen, it can utilize ammonia, nitrate, nitrite, urea, peptone, and asparagin. It is autotrophic only when, in the absence of organic carbon, it can not develop heterotrophically.

Kaserer's discovery that *B. pantotrophus* is capable of producing formaldehyde out of hydrogen and carbon dioxid, and of further using the formaldehyde thus formed as a source of energy, was almost accidental. In order to prevent the drying out of the silica plates, he placed under the bell jar a small vessel containing distilled water. Large numbers of bacteria were found to develop in this distilled water to such an extent as to cause the oxidation of the hydrogen before the colonies could develop on the plates. Kaserer added a few drops of a silver nitrate solution to the distilled water, in order to keep out the invaders. To his astonishment, he noted the deposition of a silver mirror on the walls of the vessel, a reaction given by aldehydes. Control experiments showed that the mirror was formed also when the silica jelly plates contained calcium carbonate, precluding thus the escape of formic acid. This pointed strongly to the formation of formaldehyde, whose amount increased when there were vigorously growing colonies on the silica plates. Further experiments proved that free formaldehyde is actually present in liquid cultures of *B. pantotrophus*, and also that the latter can stand comparatively large amounts of formaldehyde in the culture medium (1–15,000). Kaserer observed, further, that *B. pantotrophus* is unable to oxidize hydrogen in the presence of organic substance, pre-

ferring under those conditions to make use of the latter as a source of energy. On the other hand, such organic substances as cellulose, which are insoluble and can not therefore serve as food to the organism, do not interfere with the oxidation of the hydrogen. It may also be added here that *B. pantotrophus* prefers ammonia to nitrate as a source of nitrogen when living autotrophically.

Beijerinck and Van Delden reported[a] in 1903 the isolation of an organism which they named *B. oligocarbophilus*, and which they believed had already been encountered by Heraeus as early as 1886.[b] At any rate the latter noted the increase of bacteria in purely inorganic media until there was a very considerable accumulation of organic substance. These authors likewise report in a footnote that they had studied also another organism similar in its properties to *B. oligocarbophilus*, but belonging to the Streptothrix group. *B. oligocarbophilus* was isolated from inorganic solutions, containing phosphorus, potassium, sodium, magnesium, sulphur, manganese, iron, chlorin, and nitrogen. When nitrogen, phosphorus, potassium, or magnesium were purposely omitted from the nutrient solution there was either no growth at all or only very slight growth. The culture medium, when inoculated with garden soil and kept in the dark at 23–25° C., shows in three weeks a thin, white, dry membrane, made up of very small bacilli embedded in a pectin-like material. *B. oligocarbophilus*, in pure cultures, did not form nitrates from ammonia salts and nitrates; hence, it is not a nitrifying organism. Exclusion of air prevented growth, while in flasks with narrow necks it was meager. Evidently there was some carbon compound in the atmosphere which served as a source of carbon to the organism. The attempts made by Beijerinck and his associates to determine the nature of this carbon compound were not successful. They demonstrated, however, that it was not carbon dioxid, for *B. oligocarbophilus* could not utilize it for its growth, and they were led to assume that the organism subsisted on a certain organic constituent of the atmosphere first discovered by Karsten in 1862,[c] rediscovered more recently by French investigators, particularly Henriet,[d] and assumed by the latter to be a substituted formamid, HCO.NHR. The air of the laboratory apparently contained a larger proportion of this compound, since it allowed a more or less extensive growth, whereas no appreciable growth took place in the greenhouse. The authors conclude finally that the activities of the organism discovered by them bear a certain relation to the biological purification of the atmosphere analogous to the purification of streams by water bacteria.

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[a] Centbl. Bakt. [etc.], 2. Abt., 10 (1903), p. 33.

[b] Ztschr. Hyg., 1 (1886), p. 226.

[c] Ann. Phys. u. Chem. [Poggendorff], 115 (1862), p. 343.

[d] Compt. Rend. Acad. Sci. [Paris], 135 (1902), p. 101; 136 (1903), p. 1465.

Impure cultures of *B. oligocarbophilus* were probably studied also by Volpino,[a] who ascribed to it the power of securing not only its carbon, but also its nitrogen from the corresponding compounds in the air. The organism failed to multiply in air freed from ammonia. Kaserer,[b] who worked with pure as well as with crude cultures of *B. oligocarbophilus,* observed that only the latter could oxidize hydrogen. The oxidation of hydrogen by pure cultures was accomplished in the presence of *B. methylicus* Loew, or of other organisms. On the other hand, pure cultures of *B. oligocarbophilus* were found capable of oxidizing carbon monoxid. This remarkable property, not hitherto ascribed to any living organism, is in full accord with the observations of Beijerinck and Van Delden. The better growth of pure cultures in the laboratory air, as noted by them, was apparently due to its greater content of carbon monoxid. "The fact," says Kaserer, "that carbon monoxid can be oxidized chemically by atmospheric oxygen, as well as biologically by universally occurring microorganisms, makes the scarcely justifiable assertion of Bottomley and Jackson[c] that green plants can assimilate carbon monoxid, seem even more doubtful, for the carbon monoxid may disappear in one or both of these ways, and the carbon dioxid formed may be assimilated by the plants.

Kaserer would divide the organisms capable of oxidizing hydrogen into two groups, each accomplishing the result in its own way:

$$CO_2+H_2O+2H_2=CH_2O+2H_2O$$
$$CO_2+H_2O+H_2=CO+2H_2O$$

These reactions correspond to the characteristics of the two organisms, one of them, *B. pantotrophus,* being a representative of the carbohydrate world; the other, *B. oligocarbophilus,* being the representative of the carbon monoxid world. The autotrophic organisms, hitherto discovered, appear to belong to the carbon monoxid world, and show for this reason a very remarkable aversion to organic substances. The assumption that the autotrophic nitrite and nitrate bacteria utilize carbon monoxid as the first assimilation product finds support in the fact that the oxidation of ammonia to nitrate occurs in two phases:

$$\begin{array}{l} \quad H \\ \quad H \\ ONH \\ \quad H+O=CO+HNO_2+2H_2O-2\ \text{Cal.} \\ \ \ CO\quad O \\ \ \ OH \\ (199\ \text{Cal.})\ (29\ \text{Cal.})\quad (30\ \text{Cal.})\quad (2.69\ \text{Cal.}). \\ 2\ CO+O_2+2H_2O=2H_2CO_3+148\ \text{Cal.} \\ (2.29\ \text{Cal.})\quad (2.69\ \text{Cal.})\quad (2.172\ \text{Cal.}). \end{array}$$

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[a] Riv Ig. e Sanita Pub [Rome], 16 (1905), p. 587; abs. in Centbl. Bakt. [etc.], 2. Abt. 15 (1905), p. 70.

[b] Centbl. Bakt. [etc.], 2. Abt., 16 (1906), p. 695.

[c] Proc. Roy. Soc. [London], 72 (1903), p. 130.

The hypothesis is thus made here that ammonium acid carbonate in water solution is dissociated in the presence of oxygen into carbon monoxid and nitrous acid, the process being facilitated by the nitrous ferment in its utilization of the carbon monoxid as a source of carbon and of energy. In support of this hypothesis it will be necessary to prove that the nitrous ferment can subsist like *B. oligocarbophilus* on carbon monoxid.

The reaction for the nitrate ferment would be:

$$\begin{array}{l}
\mathrm{OH} \\
\mathrm{CO+NOH=CO+H_2O+HNO_3-55\ Cal.;\ 2CO+O_2+2H_2O=2H_2CO_3+148\ Cal.} \\
\mathrm{OH\quad\ O} \\
\mathrm{(172\ Cal.)\quad (30\ Cal.)\quad (29\ Cal.)\quad (69\ Cal.)\quad (49\ Cal.).}
\end{array}$$

Also, in this instance, it is necessary to assume that energy is derived from the carbon monoxid, and that the nitric ferment is capable therefore of utilizing the latter for its life processes.

Kaserer states that if his hypothesis as to the existence of two groups of autotrophic organisms is correct, then there is great probability that there is an organism which on the one hand can produce the reaction:

$$\begin{array}{l}
\quad\mathrm{H} \\
\quad\mathrm{H\qquad\ H} \\
\mathrm{ONH+O=CO+HNO_3+H_2O-41\ Cal.} \\
\quad\mathrm{H\quad O\quad H} \\
\mathrm{CO} \\
\mathrm{OH} \\
\mathrm{(199\ Cal.)\ (40\ Cal.)\quad (49\ Cal.)\quad (69\ Cal.).} \\
\mathrm{CH_2O+O_2=H_2CO_3+132\ Cal.}
\end{array}$$

That is, it can directly transform ammonia into nitrate, and is, on the other hand, nonsusceptible to formaldehyde and can grow on gelatin. Kaserer claims further that he has actually secured impure cultures of such an organism. In so far as carbon assimilation by green plants is concerned, there is, according to him, a photolysis of carbon dioxid as well as of water by the chlorophyl, oxygen is split off, the hydrogen unites with carbon dioxid under the influence of a formaldehyde-forming enzym to form formaldehyde and water; finally, the formaldehyde is condensed into the higher carbohydrates.

Nabokich and Lebedeff,[a] who repeated Kaserer's experiments under more exact experimental conditions, confirmed his results. Their purely mineral culture solutions were kept in an atmosphere of hydrogen and oxygen to which a small proportion of carbon dioxid had been added. After twenty-five or thirty days an almost complete vacuum was usually found in the culture vessels, the three gases having been used up by the bacteria. The authors concluded, therefore,

[a] Centbl. Bakt. [etc.], 2. Abt., 17 (1906), p. 350.

that they had succeeded in demonstrating unequivocally that hydrogen may be oxidized by autotrophic rod-shaped bacteria.[a]

The reactions noted above concern the organic constituents of soils and deal prominently with one of the elements of plant food—nitrogen. The several processes of ammonification, of nitrification, of denitrification, and of nitrogen fixation possess to us a paramount interest because they concern this very element whose fortunes are so closely allied to the activities of soil bacteria. We have learned to know that small harvests are more frequently due to lack of nitrogen than to lack of potassium or of phosphorus; we have also learned to know that such lack of nitrogen is affected in one direction or another by hungry hosts of soil organisms. Seeking in nitrogen a portion of their food, or even of energy for their life processes, they multiply the number of its combinations, and compel it to pass through a series of synthetical or analytical changes. The nitrogen atom, to-day a constituent part of some complex protein molecule, may to-morrow be made a part of some simple amido acid, or of an ammonia molecule, or may unite with another nitrogen atom and pass into the overlying air. We know that crops depend on the soil bacteria for a proper supply of nitrogen in their food, and it can not be a matter of indifference how the soil organisms affect the transformation of the insoluble and inaccessible (to higher plants) humus nitrogen into the readily diffusible and assimilable nitrate nitrogen. It can not be a matter of indifference whether smaller or greater losses of nitrogen occur in the economy of nitrogen transformation by the soil bacteria. Nor can it be a matter of indifference whether those soil bacteria that are capable by themselves or in symbiosis with other organisms of laying hold of the gaseous elementary nitrogen and of compelling it to unite with other elements, find in the soil conditions favorable or unfavorable to their development. It is because of the intimate relation of soil bacteria to soil nitrogen, and the frequently controlling position of soil nitrogen as a factor in crop production, that there is such a strong interdependence of soil bacteria, soil nitrogen, and soil fertility. The knowledge of these facts has been productive of extended research, of which by far the greater part has been devoted to the study of soil bacteria from the standpoint of soil nitrogen, and, as already noted, most of the attempts to designate the work of soil bacteria in terms of soil fertility have been based on this very point of view.

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[a] Centbl. Bakt. [etc.], 2. Abt., 17 (1906), p. 355.

TRANSFORMATION OF MINERAL CONSTITUENTS OF SOILS.

It does not follow, however, that the soil bacteria bear but an unimportant relation to the so-called mineral elements of plant food, to the potassium, phosphorus, magnesium, calcium, iron, sulphur, or even silicon, in the soil. Indeed, there is reason to believe that soil bacteria influence soil fertility through their relations to these several elements, yet these relations are to us largely unknown. Theoretically, at least, there is good reason to suppose that the phosphates in the soil are to a considerable extent affected in their solubility by the products of bacterial activity. Remembering that the production of carbon dioxid is, in a measure, an index of the intensity of the decomposition processes in the soil humus, and remembering also that carbon dioxid plays a very important rôle in the weathering of rock fragments, we receive a hint as to the possible influence of the decay bacteria in rendering available the mineral constituents of plant food. This point is illustrated by the following reaction:

$$Ca_3(PO_4)_2+2CO_2+2H_2O=Ca_2H_2(PO_4)_2+Ca(HCO_3)_2.$$ [a]

It is possible, too, that the solution of calcium bicarbonate thus formed, together with that furnished by the calcium carbonate in the soil, may react upon such silicates as orthoclase and liberate their potash as carbonate, the lime uniting with the other constituents of the mineral, thus:

$$Al_2O_3.K_2O.6SiO_2+Ca(HCO_3)_2=Al_2O_3.CaO.6SiO_2+2KHCO_3.$$

The soluble potassium salt is then either absorbed by the roots of the crop growing on the soil, or held in a weak state of combination by the hydrated silicates present.

The breaking down of the soil humus by bacteria is accompanied by the formation of various organic compounds, notably organic acids, which react with some of the constituents of the insoluble mineral fragments. Grandeau[b] was of the opinion that there are double compounds formed from the organic and mineral portions of the soil, and that these double compounds are largely instrumental in increasing the fertility of cultivated soils.

Wollny[c] says the indirect action of the humic constituents is to a great extent due to the fact that the compounds formed in their decomposition can effect the solution of the various undissolved

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[a] Ingle. Manual of Agricultural Chemistry. London: Scott, Greenwood & Co., 1902, p. 59.

[b] Jour. Agr. Prat., 36 (1872), I, pp. 435, 471, 506, 542, 581, 685; II, pp. 577, 649, 685, 761, 830; cited after Wollny, Die Zersetzung der organischen Stoffe. Heidelberg: C. Winter, 1897, p. 281.

[c] Wollny. Die Zersetzung der organischen Stoffe. Heidelberg: C. Winter, 1897, p. 281.

mineral constituents of the soil; and he quotes Senft[a] to the effect that silicates of the alkali metals or of the alkali earths, and the phosphates of calcium and iron are decomposed and rendered soluble when in prolonged contact with ammonium humate. The humic substances, therefore, hasten the breaking down of the mineral substances in the soil, render them assimilable, and thus exert a favorable influence on the growth of plants.

The investigations concerning the action of specific soil bacteria on the mineral constituents in the soil have not been extensive. Of the work done in this direction, there is some, however, that deserves more than a passing notice, especially that on sulphate reduction. Beijerinck[b] found in garden soil and elsewhere an organism which he named *Spirillum desulfuricans*, and which Van Delden later classified as *Microspira desulfuricans*.[c] Beijerinck showed this organism to be capable of reducing various sulphates with but slight increase of organic substance. Another sulphate-reducing organism (*M. æstuarii*) was isolated by Van Delden,[d] who compared the two under various conditions, and believes he has proved that the reduction of sulphates by *M. desulfuricans* and *M. æstuarii* is a process possible only under anaerobic conditions, and in a medium containing besides the sulphates also some suitable organic nutrient.

These experiments also showed clearly that a large variety of organic compounds may be oxidized by means of the sulphate oxygen, and it appears definitely that this oxygen may act like nitrate oxygen in the self-purification of streams and in the biological purification of sewage. Denitrification which was more intimately studied in this direction by Van Iterson, finds thus a biological analogy in sulphate reduction.

It is not known to what extent sulphate reduction in the soil affects soil fertility and crop production, but the mere presence of these organisms in the soil would indicate that they have some function to perform there. Moreover, it would seem that the reduction of sulphates in the soil is not limited to the two Microspira or to others like them. Some interesting experiments by Nadson[e] show that both *Proteus vulgaris* and *Bacillus mycoides*, organisms seldom absent from arable soils, can produce hydrogen sulphid on organic media, the amount produced being increased on the addition of calcium sulphate, and consequent upon the reduction of the latter. Such

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[a] Lehrbuch der Gesteins-und Bodenkunde. Berlin, 1877, 2. ed., p. 330.

[b] Centbl. Bakt. [etc.], 2. Abt., 1 (1895), p. 1.

[c] Ibid., 11 (1903–4), p. 81.

[d] Ibid.

[e] Die Mikroorganismen als geologische Faktoren. I. Ueber die Schwefelwasserstoffgährung im Weissowo-Salzsee und über die Betheiligung der Mikroorganismen bei der Bildung des schwarzen Schlammes (Heil-Schlammes). (Text in Russian.) St. Petersburg, 1903, p. 79.

reduction does not seem to take place, however, when air is freely admitted to the culture, and Nadson concludes that oxygen is thus withdrawn from calcium sulphate or other sulphates when the atmospheric supply is limited. He would explain the process by the action of nascent hydrogen on the sulphate in the presence of carbon dioxid and ammonia forming ammonium sulphate, which is in turn acted upon by nascent hydrogen with the evolution of hydrogen sulphid.

The hydrogen sulphid formed in the soil is again likely to pass through a series of transformations, involving the activities of two or three groups of very interesting bacteria, some of which were studied by Winogradski.[a] The potential energy of the hydrogen sulphid molecule is transformed into available energy by the oxidation of the hydrogen and the deposition of elementary sulphur in the bacterial cell, and later by the oxidation of the sulphur itself with the formation of sulphuric acid and of sulphates. In this connection, Fische[b] says, the significance of the sulphur bacteria for the great cycle of transformation of matter in nature lies in the fact that they change the sulphur of the hydrogen sulphid, of itself unavailable to green plants, into sulphates that are easily absorbed, and thus make accessible for the building anew of living cells, a product uniformly appearing in the decay of dead organisms.

Another set of interesting reactions that may occur in the soil is pointed out by Nadson and deserves mention here. According to him[c] soil bacteria may also cause the formation of calcium carbonate by means of ammonium carbonate formed in the decay of protein substances:

$$(NH_4)_2CO_3+CaSO_4=CaCO_3+(NH_4)_2SO_4$$

by the reduction of calcium sulphate, the formation of calcium sulphid and the action on the latter of carbon dioxid in the presence of water:

$$CaS+CO_2+H_2O=CaCO_3+H_2S.$$ [d]

He also observed the formation of calcium carbonate in decomposing organic compounds (gelatin, peptone, etc.), containing calcium, and assumes that bacteria must also play an important part in the cycle of transformation of magnesium, since the latter usually accom-

[a] Ueber Schwefelbacterien. Bot. Ztg., 45 (1887), pp. 489, 513, 529, 545, 569, 585, 606; abs. in Ann. Inst. Pasteur, 1 (1887), p. 548. See also Conn, Agricultural Bacteriology. Philadelphia: P. Blakiston and Son, 1901, p. 59.

[b] Vorlesungen über Bacterien. Jena, 1903, p. 141.

[c] Die Mikroorganismen als geologische Faktoren. I. Ueber die Schwefelwasserstoffgährung im Weissowo-Salzsee und über die Betheiligung der Mikroorganismen bei der Bildung des schwarzen Schlammes (Heil-Schlammes). (Text in Russian.) St. Petersburg, 1903, p. 84.

[d] Murray and Irvine, Trans. Roy. Soc. Edinb., 37 (1893), pt. 2, p. 496.

panies calcium. In fact, he demonstrated the formation of dolomite, or at least of a mixture of calcium and magnesium carbonates in certain bacterial changes, and noted the formation of these carbonates in a medium inoculated with a pure culture of *Proteus vulgaris*. No such action was observed in the sterile control solutions.

The more recent investigations of Hall and Miller[a] bring additional evidence in accord with that of Nadson. In trying to account for the renewal of basic substances, and particularly of calcium carbonate from the soil, they came to the conclusion that there must be compensating factors in arable lands which help to restore somewhat the rapid wasting of the carbonates of calcium and magnesium. It was shown in their investigations that the soils of Rothamsted are losing lime at the rate of 800 to 1,000 pounds per acre annually, and that certain plats receiving applications of artificial manures suffer increased losses of lime as compared with the unmanured soils. This fact is not difficult to explain, since the substances thus introduced place a further drain upon the calcium carbonate by the reactions which they involve.

It is well known that considerable quantities of lime are removed from all soils as calcium bicarbonate. Cavendish,[b] who discovered that the carbonate is soluble in water charged with carbon dioxid, demonstrated the presence of the bicarbonate in many natural waters, and indicated thus a serious source of loss from the calcium carbonate stores of the soil.

Johnston wrote in 1849:[c]

> Experience everywhere teaches that the influence of the lime we have laid upon our fields after a time gradually diminishes, the grass becomes visibly less rich and fuller of weeds every year, the crops of corn less abundant, the sample of grain inferior, and the kind of grain which the land will ripen less valuable. Does the lime actually disappear from the soil, or does it merely cease to act? This question was most distinctly answered by an experiment of Lampadius.[d] He mingled lime with the soil of a piece of ground until it was in proportion of 1.19 per cent of its whole weight, and he determined subsequently by analysis the proportion of lime it contained in each of the three succeeding years. The first year it contained 1.19 per cent carbonate of lime; the second, 0.89 per cent; the third, 0.52 per cent; the fourth, 0.24 per cent. There can be no question, therefore, that the lime gradually disappears or is removed from the soil.

We see thus that the extent of the loss of lime from soils was fully appreciated as far back as the middle of the last century. It was also well known at that time that the removal of calcium bicarbonate

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[a] Proc. Roy. Soc. [London], Ser. B, 77 (1905), No. B 514, p. 1.

[b] Cited after Hall and Miller. Ibid., p. 1.

[c] Use of Lime in Agriculture. Edinburgh and London: Blackwoods, 1849, p. 179. See also Johnston. Lectures on the Applications of Chemistry and Geology to Agriculture. New York: Wiley and Putnam, 1847, p. 398.

[d] Schübler, Agricultur Chemie, II, p. 141.

in the drainage waters went far toward accounting for this loss. Johnston [a] also says:

The water that flows from the drains in our own cultivated fields is almost always impregnated with lime, and sometimes to so great a degree as to form calcareous deposits in the interior of the drains themselves where the fall is so gentle as to allow the water to linger a sufficient length of time before it escapes into the nearest ditch or brook. * * * This effect of the rain in washing or dissolving out the lime of the soil is so marked that it is no uncommon thing to find soils which rest immediately upon limestone or even upon soft chalk rock almost entirely destitute of lime. * * * In chalk soils this comparative absence of lime from the upper soil has been frequently observed, and it serves to explain what at first sight appears surprising, that the chalking of such land should be so prevailing a practice and that it should be attended with such generally beneficial effect.

The analyses made by A. Voelcker [b] and Frankland [c] of the drainage waters from the Broadbalk wheat field at Rothamsted showed the presence of 99 parts per million of CaO in the drainage waters from the unmanured plats, of which 84 parts existed as bicarbonate. Similarly, the drainage waters from the plat receiving annual applications of farmyard manure contained 147 parts of CaO per million, of which 72 parts were probably in the state of bicarbonate. "While such losses," say Hall and Miller, "may be regarded as natural, it has been long known that many of the substances applied to the soil under the general terms of artificial manures react with the calcium carbonate there present and bring about its dissolution." The following reactions illustrate this point:

$$CaH_4(PO_4)_2+2CaCO_3=Ca_3(PO_4)_2+2H_2O+2CO_2$$
$$(NH_4)_2SO_4+CaCO_3=(NH_4)_2CO_3+CaSO_4$$
$$2KCl+CaCO_3=K_2CO_3+CaCl_2$$
$$(NH_4)_2SO_4+2CaCO_3+4O_2=Ca(NO_3)_2+CaSO_4+4H_2O+2CO_2$$

In farm practice involving the extensive use of artificial fertilizers the loss through the reactions indicated may be quite considerable. On the whole, however, it is the removal of the bicarbonate which constitutes by far the greatest source of loss. The formation of carbon dioxid in the decomposition of organic matter is essentially a biological process, hence the removal of calcium bicarbonate from the soil bears a certain relation to its bacteriological activities. The part played in this process by decomposing organic matter was recognized years ago. We may again quote Johnston in this connection:

The ultimate resolution of all vegetable matter into carbonic acid and water, which is its tendency in the soil, likewise aids the removal of the lime. For if the soil be everywhere impregnated with carbonic acid, the rain and spring waters that flow through it will also become charged with this gas, and thus be enabled to dissolve a larger portion of the carbonate of lime than they could otherwise do. Thus theory

[a] l. c., pp. 181, 182.

[b] Jour. Chem. Soc. [London], 24 (1871), p. 276.

[c] Jour. Roy. Agr. Soc. [London], 2. ser., 18 (1882), p. 14.

indicates what I believe experience confirms—that a given quantity of lime will disappear soonest from a field, if under arable culture, in which animal and vegetable matter is most abundant.

Johnston might have pointed out that the reaction is in a manner reciprocal. Larger amounts of organic matter hasten the removal of calcium carbonate from the soil, while larger amounts of calcium carbonate encourage the development of most of the soil bacteria and thus stimulate the production of carbon dioxid. Different species of soil bacteria show marked variations in their ability to produce carbon dioxid from the soil humus. Furthermore, much is yet to be learned of the conditions which modify the activities of these organisms. It will readily be seen, at any rate, that the great importance of carbon dioxid as affecting the availibility of the phosphorus and potassium compounds in the soil makes it highly desirable that the bacteriological factors concerned in its production be given careful study in the future. One need but to recall here the rapid wasting of the humus in the prairie soils, the vast significance of green manures and the conditions affecting their decomposition, and the value of untreated phosphates in the presence or absence of rapidly decomposing organic matter. The evidence is therefore overwhelming that soil bacteria play a predominant part in the removal of calcium carbonate from the soil, and incidentally influence also the availability of the potassium and phosphorus compounds in the rock fragments. Yet this is but one side of the process. There are, as was stated above, also compensating factors which provide for a partial restoration, at least, of the enormous amounts of calcium carbonate removed.

It would be worth while to remember, in this connection, that the nitrates of sodium, potassium, magnesium, and calcium leave basic residues in the soil, since the nitric acid radicle is used in greater proportion by the growing plants than is the base. As Hall and Miller point out:[a]

The plats receiving sodium nitrate in place of ammonium salts show not only no special loss of calcium carbonate due to the nitrogenous manure, but a distinctly diminished loss as compared with the unmanured plats. * * * Where the lime is used there is both a lower concentration of lime in the drainage water and a smaller total percolation, because of the much greater crop, and consequently increased transpiration. The sodium nitrate then either saves the calcium carbonate of the soil from its normal loss or has some power to bring about the re-formation of calcium carbonate.

In speaking of the plats receiving farmyard manure, the authors say:

The drain beneath the farmyard manure plat on the Broadbalk field runs but rarely, because the humus derived from the long-continued organic manuring of this plat is capable of temporarily absorbing any ordinary rainfall and then passing it gradually down to the subsoil without causing the drain to run. But the few analyses

[a]Proc. Roy. Soc. [London], Ser. B, 77 (1905), No. B 514, p. 19.

that have been made of the water draining from this plat indicates a lower concentration in calcium compounds than would be expected from the large amount of carbon dioxid produced by the decay of recent organic matter, and also from the considerable annual addition of calcium compounds in the manure itself. * * * We thus obtain three lines of evidence that there is some agency saving or re-creating the calcium carbonate in the soil: (1) The loss of calcium carbonate induced by the use of ammonium salts is less than half that required for the absorption and subsequent nitrification of the ammonia; (2) where sodium nitrate or (3) where farmyard manure is applied, the rate of loss of calcium carbonate is below that of unmanured land.

Further evidence that there must be under normal conditions some action at work protecting or renewing the bases of the soil may be gathered from the continued fertility of many soils containing but a trace of calcium carbonate.

Hall and Miller then give the calcium carbonate content of a number of soils which continue to be fertile, notwithstanding the very low proportion of this compound, and speaking of the Stackyard field on the farm of the Royal Agricultural Society at Woburn, which has been under experiment since 1876, they say:

The amount of calcium carbonate present is exceedingly small, barely determinable in fact, yet the plats continue to yield normal crops, except those which have been manured with ammonium salts. The latter in recent years have become almost sterile, showing an acid reaction to litmus paper and refusing to grow wheat or barley unless they first receive a dressing of lime.

Now in all these cases, however low the proportion of calcium carbonate may be, the action of the percolating water must remove some of it, and the recurring process of nitrification also demands a base. Yet the small quantity of base available does not disappear entirely, so as to render the soil unfertile, unless some specially calcium carbonate consuming material, like the ammonium salts, is employed as a manure. The continued fertility of such soils almost devoid of calcium carbonate has long been a problem, but it now seems probable that the calcium carbonate and other bases, which are required for nitrification, are in some way restored to the soil as bases, and that when a ready-formed nitrate like sodium nitrate is used as manure there is an addition of available base to the soil or a corresponding diminution in the amount of calcium carbonate removed by the drainage water. Furthermore, these or other agencies conservative of calcium carbonate are sufficient to maintain the quantity in the soil at the level for comparatively healthy growth. Of the positive conservative actions, two will be now considered and evidence be brought to show that (1) the normal growth of plants leaves behind a residue of base in the soil; (2) the decay of plant tissues results in the production of calcium carbonate.

Hall and Miller attempt to prove the first part of their thesis as follows:

When a plant is burned, the ash is usually alkaline, because the organic acids and any nitrogen present as nitrate in the plant are all driven off, leaving the bases as carbonates. But when a balance is struck between the acids and bases in the ash and when the nitrogen present in the plant before burning is calculated as an acid, since it all entered the plant as nitrate, the acids are generally to be found in excess. Warington,[a] indeed, has already pointed out with reference to the published analyses, that plants must retain more acids than bases. It does not appear to have been noticed, however, that such a result, by leaving behind in the soil a corresponding excess of base, must have an appreciable effect upon the reaction of the soil, although Knop

[a] Agr. Students' Gaz., n. ser., 9 (1899), p. 133.

and other early investigators have observed that the solutions in which plants are grown as water cultures become alkaline after a time. * * * The normal growth of farm crops leaves behind from the salts in the soil used for its nutrition about as much base as would have been previously required for the nitrification of the nitrates which entered the plant, as measured by the nitrogen finally contained in it. * * * The results, as a whole, go to show that the action of plants, in leaving behind a basic residue from the neutral salts in the soil upon which they feed, is a very essential feature in the chemistry of the soil, explaining, amongst other things, the maintenance of healthy conditions on the many soils poor in calcium carbonate.

Turning now to the second part of their thesis as of more direct interest here, since it concerns the activities of soil bacteria, we note that their investigations include also some studies calculated to prove that calcium salts of organic acids may be converted by soil organisms into calcium carbonate. Their results, they believe, "serve to show that the soil contains one or more organisms which are very effective in converting calcium oxalate into carbonate." Mention is also made by them of the basic residues left by denitrifying bacteria, although it is scarcely probable that this is of much practical significance in soils. They might have added here that also the nitrogen-fixing organisms of the Azotobacter group, and many other bacteria still to be studied in this connection, leave basic residues in the soil. On the whole they have much reason for the following conclusions:

Doubtless in all soils containing only a minimal amount of calcium carbonate under natural conditions these various actions have reached an equilibrium, since the increase of any one only tends to bring into play the factor which limits it (the rate of nitrification, for example, will be slowed down as the available base in the soil becomes scarce), but also accelerates the operation of some action in the opposite sense; even the one irrevocable loss by drainage and removal of crop will probably be balanced by the calcium salts covering into solution through the continued weathering of the soil particles. In the main, however, the original stock of calcium carbonate in the soil circulates continually between plant and soil without suffering appreciable loss. It is only under particular conditions, such as the use of ammoniacal manures, or the setting up of anaerobic conditions through lack of drainage, thus allowing the formation of organic decay acids but not their final oxidation to carbonates, that the soil will develop an acid reaction and become infertile.[a]

The restoration of calcium carbonate by plant and bacterial activities in the soil, as pointed out by Hall and Miller, is undoubtedly of considerable practical significance. There is one phase of the subject, however, to which these authors have apparently failed to accord a proper value. In making so much of the basic residues of plant nutrition, and likewise of the basic residues due to bacterial activities in the soil, they say scarcely anything of the decomposition of calcium silicates in the presence of carbon dioxid and the consequent formation of calcium carbonate. In this case, surely, the biological activities play an important even though indirect part. It was well known in Johnston's day that trap rocks rich in calcium silicate

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[a] See also Sedgwick, T. F. Internat. Sugar Jour., 9 (1907), p. 343.

seldom respond to applications of the carbonate, and he accounted for this circumstance by the assumption that new quantities of calcium carbonate are being formed gradually from the decaying rock. "The effect of this must obviously be to retard the natural exhaustion of the lime and to put off the period when a new dose of lime may be profitably given."

Hilgard[a] also notes that calcium carbonate may be one of the residual products in the weathering of calcium silicate. Carbon dioxid, according to him, "being easily soluble in water, whether of rains, rivers, springs, or wells, and largely of course in that percolating the soil," is a universal solvent.

> Such waters may therefore be considered as being acid solvents; and as such, they exercise a far more energetic and far-reaching effect than would pure water.
>
> While limestones are the rocks most obviously acted upon by carbonated water, few if any resist altogether. Even quartz rocks of the ordinary kinds are attacked by it; only the purest white crystalline quartzite may be considered as visibly proof against it. Granite and the rocks related to it are rather quickly acted upon, because of the presence of the feldspar minerals containing potash, soda, and lime as bases, together with alumina.
>
> The results of this action are highly important: one being the formation of clay, so essential as a physical ingredient of soils; the other the setting free of potash, one of the most essential nutrients of plants. * * * In all cases, of course, the silica set free by the carbonic acid remains partially or wholly in the resulting soils as such. Lime also at first mostly remains behind in the form of carbonate, but potash, and especially soda compounds, being mostly readily soluble in water, are largely carried away by the latter.

This statement of Hilgard shows clearly how the carbon dioxid formed by the innumerable bacterial activities of land and sea affects the decomposition of the rocks of the earth's surface, and more particularly of the rock fragments in our soils. It also demonstrates how the carbon dioxid produced by soil bacteria may hasten the accumulation of calcium carbonate on the one hand and its disappearance on the other. The factors leading to the increase or decrease of calcium carbonate in the soil do not bear a constant relation to one another, and it would seem that Hall and Miller exaggerate the magnitude of the compensating factors. Their claim that "the original stock of calcium carbonate in the soil circulates continually between plant and soil without suffering appreciable loss" does not hold good for many soils. We need but recall in this connection the investigations of the Rhode Island and Ohio experiment stations, and the quite general experience of farmers throughout the older portion of the United States. Notwithstanding the compensating factors referred to, the sources of loss still remain sufficiently great to cause the depletion of calcium carbonate in soils devoted to general farming, not to mention those given over to market gardening. Heavy

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[a] Soils. New York and London: The Macmillan Co., 1906, p. 17.

dressings of animal manures, repeated applications of acid phosphates and of potassium salts, and the frequent plowing under of green crops naturally involve extensive losses, and call for frequent compensation in form of applications of lime.

The so-called iron bacteria[a] may also bear a certain relation to soil fertility, although practically nothing is known of the nature of such relations in cultivated soils. On the other hand, the great host of decay bacteria undoubtedly play an important part in the transformation of the various iron compounds in the soil. The work of Nadson already cited contains references to the activity of *Proteus vulgaris*[b] and other bacteria in this direction. Thanks to the exclusive activity of *P. vulgaris* in pure culture, a whole series of transformations is accomplished, including that of silicon, iron, and calcium. It remains for the future investigator to shed more light on the relation of soil bacteria to the various mineral constituents of the soil, and thus make more intelligible to us some of the problems of soil fertility. As Conn[c] expresses it:

> The problem of the exact relation of soil bacteria to the mineral ingredients of the soil remains as a subject for future investigation. We know that the rocks undergo disintegration and we know that numerous chemical changes are constantly occurring in the mineral ingredients. To what extent these are explained by chemical and physical phenomena and to what extent vital forces have contributed and are constantly contributing can not to-day even be conjectured.[d]

CHEMICAL PHASES OF DECAY AND PUTREFACTION.

Putrefaction and decay were recognized as natural phenomena in prehistoric times, but their true origin did not become known until after the epoch-making discoveries of Pasteur. Like other observers, he noted[e] the essential differences in the decomposition of organic substances under varying degrees of aeration, and pointed out also that there is usually formed a thin membrane on the surface of fluids undergoing decomposition in open vessels. He claimed that it was the function of this membrane to exclude atmospheric oxygen from the interior of the liquid and to create conditions thereby favorable for anaerobic ferment action. In his opinion the decaying fluid

[a] Molisch. Die Pflanze in ihren Beziehungen zum Eisen. Jena: G. Fischer, 1892, p. 60. Adler. Centbl. Bakt. [etc.], 2. Abt., 11 (1903–4), pp. 215, 277.

[b] Die Mikroorganismen als geologische Faktoren. I. Ueber die Schwefelwasserstoffgährung im Weissowo-Salzsee und über die Betheiligung der Mikroorganismen bei der Bildung des schwarzen Schlammes (Heil-Schlammes). (Text in Russian.) St. Petersburg, 1903, p. 74.

[c] Agricultural Bacteriology. Philadelphia: P. Blackiston's Son and Co., 1901, p. 64.

[d] See also Koch, A., and Kröbe, E. Fühling's Landw. Ztg., 55 (1906), p, 225.

[e] Compt. Rend. Acad. Sci. [Paris], 56 (1863), p. 1189.

becomes the seat of two distinct chemical processes bearing a direct relation to the physiological activities of the two kinds of organisms growing in it. On the one hand, the vibrions living without atmospheric oxygen cause fermentations in the interior of the liquid—that is, they transform the nitrogenous substances into more simple, though still complex, products. On the other hand, the bacteria (or mucors) burn up these products and change them into the simplest binary compounds, like water, ammonia, and carbon dioxid.

It was generally believed at that time that decay was due to a certain micro-organism, which was designated as *Bacterium termo.* Cohn wrote in 1872 [a] that, through his own experiments, as well as those of other investigators, he was convinced that *B. termo* was the ferment of putrefaction in the same way as yeast is the alcoholic ferment, etc. He was convinced that no putrefaction could set in or proceed without *B. termo,* and he even suspected that the other bacteria, though they might, in part at least, also participate in the putrefaction processes, played here only a secondary rôle, whereas *B. termo* was the primary cause of putrefaction, the saprogenic ferment proper.

With the beginning of the eighties, bacteriologists began to think that *B. termo* was only a general name applied to the many species of rod-shaped organisms occurring in decaying substances. As Hauser stated in 1885,[b] it is a fact scientifically well established and almost generally accepted that putrefaction proper—that is, the decomposition of organic substances, and especially albuminous substances, accompanied by the evolution of ill-smelling gases, is due entirely to the presence and activity of bacteria. Nevertheless, this recognition has only served to give us a general conception of the nature of putrefaction, and the solution of the main question, fundamentally important though it be, has by no means enabled us to explain the complicated chemical and biological processes of putrefaction. For we find in every substance undergoing putrefaction, and especially in putrefying albumin, not merely a single well defined bacterial species, but a whole series of widely differing forms of which one or another may predominate at a given time, some of them always present in putrefying materials, others appearing only occasionally or at certain stages of decomposition. There is scarcely a doubt, he says further, that these different bacterial forms represent, in part at least, different kinds, even though many of these may belong to only a few distinct species.

Flügge made a clear presentation of the contemporary status of the subject in 1883 in his statement that almost impossible as it is at present

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[a] Untersuchungen über Bacterien. Beitr. Biol. Pflanz., 1 (1872), No. 2, p. 169.

[b] Über Fäulnissbacterien und deren Beziehungen zur Septicämie. Leipzig: F. C. W. Vogel, 1885, p. 2.

to express even approximately the decomposition processes of putrefaction in the form of chemical equations, we are still less able to say anything that is definite on the morphology of the agents of putrefaction. We find in putrefying substances various innumerable bacteria, but we know practically nothing that is certain as to which of these should be regarded as harmless and which of them are the real agents of fermentation; and we know nothing, likewise, as to the single reactions and phases of putrefaction processes as affected by the ferments.[a]

Hauser's isolation in 1884 and 1885 of three distinct bacterial species, capable of causing putrefactive decomposition, was a decided step in advance. Two of them, *Proteus vulgaris* (*Bactridium proteus*, *Bacillus vulgaris*, *Bacterium zopfii*), and *Proteus mirabilis*, were found capable of liquefying gelatin, the third, *Proteus zenkeri*, belonged to the nonliquefying species. Pure cultures of these organisms were found capable of effecting the rapid decomposition of meat. Cultures filtered through unglazed porcelain did not produce a similar reaction, and Hauser therefore concluded that putrefaction was not caused by chemical ferments, but through the direct activity of the bacterial cells. Hauser[b] concluded from these investigations that the bacterial species described above, not only possess a highly developed power of producing putrefaction, but that, thanks to their frequent and universal occurrence, they belong to the most important and the most common agents of putrefaction.

The first systematic study of the chemical reactions accompanying putrefactive decomposition was made by Nencki[c] in the early seventies of the last century. In artificial digestion experiments with fibrin, albumin, and gelatin he found leucin, tyrosin, and indol among the decomposition products, and also glycocol as one of the cleavage products of gelatin. He found further[d] that in the putrefaction of gelatin at 40° C. there were formed in four days to every 100 parts of the original substance 9.48 parts of ammonia, 24.2 parts of volatile fatty acids, 12.2 parts of glycocol, 19.4 parts of peptone, and 6.45 parts of carbon dioxid, in all, 71.73 per cent. The process was accompanied by a vigorous development of various organisms and the evolution of large quantities of gas. Other experiments of a similar character led Nencki to announce the general conclusion that the decomposition of albumin may be divided into two phases: (1) Hydrolysis, that is, the transformation into a readily soluble form

[a] Fermente und Mikroparasiten. Leipzig, 1883, p. 228. (Handb. Hyg. von Pettenkofer u. von Ziemssen, 1. Theil, 2. Abt., 1. Heft.)

[b] Über Fäulnissbacterien und deren Beziehungen zur Septicämie. Leipzig: F. C. W. Vogel, 1885, p. 75.

[c] Ber. Deut. Chem. Gesell.. 7 (1874), p. 1593.

[d] Ber. Deut. Chem. Gesell., 8 (1875), p. 336; Über die Zersetzung der Gelatine und des Eiweiss bei der Fäulnis mit Pankreas. Bern, 1876.

and the cleavage into amido acids; and (2) reduction and oxidation processes.

Nencki's experiments were carried on in open vessels, with free access of air. His pupil, Jeannert, on the other hand, repeated these experiments [a] with the exclusion of air, and found, among the decomposition products of gelatin, carbon dioxid, ammonia, a gas smelling like carbon bisulphid, acetic, butyric, and valeric acids, glycocol, leucin, and a collidin-base-like substance. Among the decomposition products of albumin, he found the above substances with the exception of glycocol, and also hydrogen sulphid, hydrogen, tyrosin, and amido-valeric acid. He, therefore, concludes among other things, that: (1) The decomposition of nitrogenous substances and of carbohydrates may be accomplished with the access or exclusion of air; (2) that in the latter case the decomposition is considerably less rapid, and complete decomposition requires, with exclusion of air, a period of time six times as long; (3) that the more simple chemical products formed are in the two cases identical.

The mechanism of putrefactive decomposition, the explanation of which was attempted by Nencki in 1878,[b] is analogous according to him to the purely chemical reaction between albumin and heated potassium hydroxid. There is formed in each case peptone, leucin, amido-valeric acid, carbon dioxid, ammonia, and tyrosin. When decay or fusion with potassium hydrate is prolonged, the tyrosin disappears, and there appear phenol and indol (skatol), and finally only carbon dioxid, ammonia, hydrogen sulphid, and volatile fatty acids. He believes, therefore, that since the same products are formed in the putrefactive decomposition of albumin as in its fusion with potassium hydrate, the assumption is apparently justified that in the processes of decay the rôle of the potassium hydrate is assumed by water. The latter is decomposed into hydrogen and hydroxyl, whereby the formation of oxidation and reduction products may be readily explained.

The chemistry of decay was further enriched by the investigations of the Salkowski brothers and their associates.[c] They found among the decomposition products of blood fibrin, aspartic acid, and among the decomposition products of horn and albumin, phenyl-lactic acid. In 1879 they discovered phenyl-propionic acid among the cleavage products of decaying meat. Other investigations carried on in the same year with blood fibrin, meat fibrin, fresh meat, serum albumin,

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[a] Jour. Prakt. Chem., 15 (1877), p. 353.

[b] Jour. Prakt. Chem., 17 (1878), p. 105. See also Bopp: Ann. Chem. Pharm., 69 (1849), p. 16; Engler and Janecke: Ber. Deut. Chem. Gessell., 9 (1876), p. 1411; Kühne: Ber. Deut. Chem. Gesell., 8 (1875), p. 206.

[c] Ber. Deut. Chem. Gesell., 7 (1874), p. 1050; 12 (1879), pp. 107, 648; Ztschr. Physiol. Chem., 2 (1878–79), p. 420.

and wool, showed that with the unhindered access of air *Bacillus subtilis* was always found to be present in the decaying fluids. Among the decomposition products of wool they found paraoxyphenyl acetic acid, and among those of meat, succinic, palmitic, and oleic acids. The higher fatty acids were also produced in meat previously extracted with ether. Subsequent experiments convinced them that oxy-acids of the aromatic series are always formed in putrefactive decomposition.[a] Skatol-carbonic acid was found by them in 1880, and further studies led them to the belief [b] that skatol-carbonic acid is a constant product of albumen decomposition, at least where the decomposition is more or less prolonged.

Other experiments carried on by a number of investigators added to the decomposition products certain poisonous bases which Selmi [c] designated as ptomaines. Brieger [d] prepared a whole series of such bases, only a part of which were found to possess poisonous properties. The poisonous products formed by pathogenic organisms he called toxins. Brieger observed, also, that poisonous substances are formed only in the early stages of putrefaction, and that they disappear on prolonged decomposition.[e]

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[a] Ber. Deut. Chem. Gesell., 13 (1880), p. 189.

[b] Ztschr. Physiol. Chem., 9 (1885), p. 491.

[c] Ber. Deut. Chem. Gesell., 6 (1873), p. 142; 7 (1874), p. 1642; 9 (1876), p. 195; 11 (1878), p. 808; 12 (1879), p. 297; 13 (1880), p. 206.

[d] Über Ptomaine. Berlin: August Hirschwald, 1885; Ztschr. Physiol. Chem., 7 (1882–83), p. 274; Ber. Deut. Chem. Gesell., 16 (1883), p. 1405; 17 (1884), pp. 515, 1137.

[e] See Baumann. Arch. Physiol. [Pflüger], 12 (1876), p. 67; Ber. Deut. Chem. Gesell., 10 (1877), p. 685; Ztschr. Physiol. Chem., 1 (1877–78), p. 60; 3 (1879), p. 250; 4 (1880), p. 304; 6 (1882), p. 183; 7 (1882–83), pp. 282, 553; 10 (1886), p. 123; 20 (1895), p. 583.

Baumann and Brieger. Ztschr. Physiol. Chem., 3 (1879), pp. 149, 254.

Baumann and Christiani. Ztschr. Physiol. Chem., 2 (1878–79), p. 350.

Baumann and Preusse. Ber. Deut. Chem. Gesell., 12 (1879), pp. 806, 1450, 2166; Ztschr. Physiol. Chem., 5 (1881), p. 309.

Brieger. Jour. Prakt. Chem., n. ser., 17 (1878), p. 124; Ber. Deut. Chem. Gesell., 10 (1877), p. 1027: 12 (1879), pp. 705, 1985; Ztschr. Physiol. Chem., 2 (1878–79), p. 241; 3 (1879), p. 134; 4 (1880), p. 414; 5 (1881), p. 366.

Blumenthal. Arch. Path. Anat. u. Physiol. [Virchow], 137 (1894), p. 539.

Hüfner. Jour. Prakt. Chem., n. ser., 11 (1875), p. 43.

Hoppe-Seyler. Arch. Physiol. [Pflüger], 12 (1876), p. 1; Ztschr. Physiol. Chem., 2 (1878–79), p. 1.

Jaffe. Ber. Deut. Chem. Gesell., 10 (1877), p. 1925; 11 (1878), p. 406; 12 (1879), p. 1092; Ztschr. Physiol. Chem., 2 (1878–79), p. 47; Centbl. Med. Wiss., 10 (1872), pp. 2, 481, 497.

Kühne. Ber. Deut. Chem. Gesell., 8 (1875), p. 206; Arch. Path. Anat. u. Physiol. [Virchow], 39 (1867), p. 165.

Nencki. Ber. Deut. Chem. Gesell., 8 (1875), p. 722; Jour. Prakt. Chem., 26 (1882), p. 47; Monatsh. Chem., 10 (1889), p. 506; Centbl. Med. Wiss., 16 (1878), pp. 693, 849; Arch. Expt. Path. u. Pharmakol., 20 (1886), p. 367; 28 (1891), p. 311; Ztschr. Physiol. Chem., 4 (1880), p. 371.

It was Hirschler[a] who first pointed out, in 1886, that the putrefaction of protein substances is modified by the presence of carbohydrates, The addition of various carbohydrates, glycerin, and calcium carbonate changed the decomposition of meat so that no aromatic products of putrefaction could be detected. From this he drew the conclusion that the decomposition of protein substances, when occurring outside of the animal body, and under conditions otherwise favorable, is not accompanied by the formation of the characteristic putrefaction products—indol, phenol, and oxyacids—when cane sugar, starch, dextrin, glycerin, or lactic acid are present. Winternitz,[b] who continued these experiments, attempted to discover the constituent that imparts this antiseptic action to milk, and was inclined to attribute it to the lactose. Important facts which helped to explain the difficulty were contributed by Blumenthal[c] in 1896. He demonstrated that milk to which putrefying substances were added itself underwent putrefaction only after the acids formed were gradually neutralized by the addition of soda. After all the milk sugar was used up and no more acid was formed, putrefaction of the milk set in, and he found among the decomposition products thus formed, ammonia, mercaptan, indol, skatol, phenyl-acetic acid, phenyl-propionic acid, alcohol, volatile fatty acids, and succinic acid.

The above investigations as to the chemical phases of putrefaction dealt with mixtures of various organisms. With the perfection of bacteriological methods, however, an attempt was made to study the chemical reactions as produced by single species. Thus the work of Hoppe-Seyler in 1876,[d] of Bienstock in 1884,[e] and of Hauser in 1885,[f]

Odermatt. Zur Kenntniss der Phenolbildung bei der Fäulniss der Eiweisskörper. Inaug. Diss., Leipzig, 1878; Jour. Prakt. Chem., n. ser., 18 (1878), p. 249.

Rubner. Arch. Hyg., 19 (1893), p. 136.

Salkowski, E. Ber. Deut. Chem. Gesell., 9 (1876), p. 138; 10 (1877), p. 842; 11 (1878), p. 500; Ztschr. Physiol. Chem., 1 (1877–78), p. 374; 4 (1880), pp. 55, 100; 7 (1883), p. 93; 9 (1885), pp. 23, 241; 10 (1886), p. 265; 27 (1899), p. 302.

Salkowski, E. and H. Ber. Deut. Chem. Gesell., 12 (1879), p. 653; 13 (1880), p. 2217; 16 (1883), p. 1191; Ztschr. Physiol. Chem., 7 (1883), p. 161; 8 (1884), p. 417; 9 (1885), p. 8.

Salkowski, H. Ber. Deut. Chem. Gesell., 31 (1898), p. 776.

Sécretan. Recherches sur la putréfaction de l'albumine et sur sa transformation en graisse. Inaug. Diss. [Bern]. Genève: Ramboz e Schuchardt, 1876. Also Arch. Sci. Phys. Nat., 55 (1876), p. 168.

Wälchli. Jour. Prakt. Chem., n. ser., 17 (1878), p. 71.

Weyl. Ztschr. Physiol. Chem., 1 (1877–78), p. 339.

[a] Ztschr. Physiol. Chem., 10 (1886), p. 306.

[b] Ibid., 16 (1892), p. 460.

[c] Arch. Path. Anat. u. Physiol. [Virchow], 146 (1896), p. 65.

[d] Arch. Physiol. [Pflüger], 12 (1876), p. 1.

[e] Ztschr. Klin. Med., 8 (1884), p. 1.

[f] Über Fäulnissbacterien und deren Beziehungen zur Septicämie. Leipzig: F. C. W. Vogel, 1885, p. 2.

concerned pure cultures, or supposedly pure cultures, of bacteria. Mention should also be made in this connection of the experiments of Nencki and Sieber,[a] Kerry,[b] Sanfelice,[c] Kühne,[d] Mörner,[e] Klecki,[f] Zoja,[g] Emmerling,[h] Bienstock,[i] Ellinger,[j] Kutscher,[k] Taylor,[l] Spiro,[m] Czapek,[n] and Ellinger and Gentzen.[o] All of these investigations carried out with aerobic and anaerobic organisms showed the formation of products analogous to those formed by mixtures of bacteria. Some of the organisms studied by them, such as *Proteus vulgaris, Bacillus subtilis, B. prodigiosus, B. putrificus, B. fluorescens liquefaciens,* and others, are well known inhabitants of the soil, and it is interesting to note that ammonia was one of the constant products of transformation induced by them in the decomposing substances.

Bienstock pointed out also that certain aerobic and facultative anaerobic organisms seem to encourage putrefaction by creating conditions favorable for the growth of anaerobic species. An exception to this case is formed by the bacteria of the intestinal tract, particularly *Bacterium coli,* as well as *B. aerogenes.* These retard or suppress the development of the anaerobic organisms of putrefaction. Bienstock found in the case of milk that when the latter was sterilized and then inoculated with obligate anaerobes it underwent putrefactive decomposition like albuminoid substances, whereas no such change took place in nonsterilized milk. According to him, therefore, the resistance of milk to putrefactive decay is due to the acid-forming aerobic or facultative anaerobic species, notably *B. aerogenes.* He actually proved by experiments that sterilized milk, inoculated with certain anaerobic organisms, underwent putrefactive decomposition, but that such decomposition did not occur when *B. coli* or *B. aerogenes* were also introduced into the sterile milk, together with the anaerobic organisms in question. It is

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[a] Monatsh. Chem., 10 (1889), p. 526.

[b] Ibid., 10 (1889), p. 864.

[c] Atti Accad. Med. Roma, 16 (1891), p. 379.

[d] Ztschr. Biol., 29 (1892), p. 1; 30 (1894), p. 221.

[e] Ztschr. Physiol. Chem., 22 (1896–97), p. 514.

[f] Centbl. Bakt. [etc.], 2 Abt., 2 (1896), pp. 169, 249, 286.

[g] Ztschr. Physiol. Chem., 23 (1897), p. 236.

[h] Ber. Deut. Chem. Gesell., 30 (1897), p. 1863; 35 (1902), p. 2289.

[i] Arch. Hyg., 36 (1899), p. 335; 39 (1901), p. 390.

[j] Ber. Deut. Chem. Gesell., 31 (1898), p. 3183; 32 (1899), p. 3542; Ztschr. Physiol. Chem., 29 (1900), p. 334.

[k] Ztschr. Physiol. Chem., 32 (1900), p. 419.

[l] Ibid., 36 (1902), p. 487.

[m] Beitr. Chem. Physiol. u. Path. [Hofmeister], 1 (1901), p. 347.

[n] Ibid., (1902), p. 538; 2 (1902), p. 557; 3 (1902), p. 47.

[o] Ibid., 4 (1903), p. 171.

thus clear that the presence of carbohydrates and of acid-forming aerobic and facultative anaerobic bacteria changes the character of decomposition, which nitrogenous materials undergo, and it may be remarked here, in passing, that *B. coli*, a normal inhabitant of the intestinal tract, probably guards the system against the injurious work of the putrefactive organisms, at least in the somewhat better aerated zone immediately adjacent to the intestinal wall.

Practically all of the observations noted above were made on nitrogenous substances of animal origin. It had already been pointed out by Bienstock that the same decomposition products are also formed in the bacterial transformation of vegetable protein bodies. This was confirmed by the experiments of Olig[a] on the decomposition of cotton-seed meal. The latter found that when air is excluded there develop sugar-fermenting and gas-producing, rod-shaped bacteria, of the coli type, and also spherical organisms, capable of fermenting sugar without the production of gas. A number of neutral species also appear. Obligate anaerobes do not develop under ordinary conditions in cotton-seed meal, and are probably suppressed by the acid-forming, sugar-fermenting species. The development of the bacteria is always accompanied by considerable losses of organic substance. Where air is excluded, or only sparingly admitted, the losses fall most heavily on the carbohydrates. Later on the protein substances and pentosans are attacked more vigorously. The fat is changed but slightly. The crude fiber is at first increased, and subsequently again diminished. The protein-decomposing bacteria of the cotton-seed meal destroy animal and vegetable proteids in the same way. There may be formed among the cleavage products: Albumoses, peptones, amin bases, volatile acids of the fatty-acid series (such as butyric and valeric), aromatic acids (phenol-acetic and phenyl-propionic), succinic acid, skatol carbonic acid, aromatic oxyacids, indol, skatol, phenol, as well as kresol, and also ammonia, carbon dioxid, and volatile compounds containing sulphur.

The chemical reactions of decomposition outlined here are of extreme importance to plant and animal life, for it is through them that the carbon, hydrogen, and nitrogen previously held fast in organic combinations are again brought into circulation. In so far as the nitrogen is concerned, the decomposition processes may be quite wasteful in that disproportionately large amounts of it may escape from its combinations, and be returned as nitrogen gas to the atmosphere. This phase of the nitrogen question is again referred to in the discussion of denitrification processes (p. 68). Meanwhile it would be well to consider here that phase in the simplification of

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[a]Die Zersetzung pflanzlicher Futter und Nahrungsmittel durch Bakterien. Inaug. Diss., Münster [Berlin: Julius Springer], 1903.

nitrogenous organic matter in the soil, where ammonia is split off from its compounds. This phase in the transformation of nitrogen is designated by the general term "ammonification."

AMMONIFICATION.

Ammonification, or the production of ammonia in the decomposition of nitrogenous organic substance, is a very important phase in the cycle of transformation to which nitrogen is subject. It represents a certain stage in the process of simplification, and bears an intimate relation to soil-fertility conditions. The chemical reactions, of which ammonia is one of the end products, are quite complicated and dependent on various biological factors that are far from constant. The moisture and temperature conditions in the soil, as well as its mechanical and chemical constitution, play a prominent rôle[a] in determining the character of the bacterial flora, and hence also the character of the chemical products formed. It is to be expected that obligate and facultative anaerobes would find fine-grained soils more to their liking, while obligate aerobes would find more suitable conditions for their survival in the better aerated, coarser-grained soils. Accordingly, we note a more extensive and vigorous flora of spore-bearing anaerobic organisms in heavy clay or loam soils than may be found under the same conditions in light sandy or sandy-loam soils;[b] differences that may be traced in the ammonifying powers of these soils. The differences thus observed are determined by the combination of species, by the proportionate and absolute numbers, and by the relative physiological efficiency of the various organisms.

It should not be supposed, however, that there are clearly defined reactions in the soil due in some cases to purely anaerobic activities, and in others to those of a purely aerobic character. Even in the most compact soils aerobic organisms do an important work, and the lightest soils are not free from anaerobes. The differences are relative rather than absolute. Bienstock [c] showed, for instance, that *Bacillus putrificus*, an obligate anaerobe, could develop under aerobic conditions and cause decomposition of fibrin, when growing together with other aerobic organisms. Such protection of anaerobic bacteria by aerobic organisms was first noted by Pasteur,[d] and is undoubtedly of great significance in the economy of soil nitrogen.

[a] Wollny. Die Zersetzung der organischen Stoffe. Heidelberg: C. Winter, 1897, p. 144.

[b] New Jersey Stas. Rpt. 1903, p. 221.

[c] Arch. Hyg., 36 (1899), p. 335.

[d] Flügge. Die Mikroorganismen. Leipzig: F. C. W. Vogel, 1896, 3 ed., Vol. I, p. 259.

In view of these facts the distinctions drawn between decay and putrefaction—that is, the decomposition of nitrogenous organic substance in the presence of oxygen on the one hand, and in the absence of oxygen (or with a limited amount of it), on the other—need not always be considered as sharply defined. With that much granted, however, it will be found that ammonification in the soil is due at times to processes partaking largely of the nature of decay, and at other times of that of putrefaction. There is at any rate a very real difference in the chemical reactions involved. Decay proper is marked by the volatilization of the organic constituents, while the nonvolatile mineral constituents are left behind in a form largely available.[a] Putrefaction denotes the rapid and intensive decomposition of nitrogenous (for the most part protein) bodies by certain bacteria, with the formation of large quantities of gaseous, ill-smelling products.[b]

The rapid oxidation of the protein bodies in processes of decay leads to the formation of carbon dioxid, water, sulphates (in the presence of a base), and ammonia. Where the oxidation processes are quite intense, a part of the ammonia is probably oxidized with the liberation of elementary nitrogen:

$$4NH_3+3O_2=6H_2O+2N_2.$$

This conclusion is justified by many recorded facts, some of which are noted elsewhere.[c] The rapid disappearance of organic matter from light, open soils may also involve losses of nitrogen through the oxidation of ammonia, although a portion of such losses may be due to the further oxidation of the ammonia to nitrates, and the subsequent leaching of the latter.[d]

In processes of putrefaction the action of the bacteria on the protein bodies is much like that of trypsin or of boiling acids. Albumoses, peptones, and amino acids are gradually formed. The latter in turn may be transformed into the corresponding simple acids with the elimination of ammonia, or carbon dioxid may be split off and diamins like cadaverin and putrescin formed. Both processes may occur simultaneously, and among others such well-known products as indol and skatol may be formed.[e] The nonnitrogenous products of putrefaction may include methane, hydrogen, hydrogen sulphid,

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[a] Wollny. Die Zersetzung der organischen Stoffe. Heidelberg: C. Winter, 1897, p. 2.

[b] Flügge. Die Mikroorganismen. Leipzig: F. C. W. Vogel, 1896, 3. ed., Vol. I, p. 254.

[c] New Jersey Stas. Rpts. 1902, p. 183; 1903, p. 217.

[d] Whitney and Cameron. U. S. Dept. Agr., Bur. Soils Bul. 23, p. 46.

[e] Cohnheim. Chemie der Eiweisskörper. Braunschweig: F. Vieweg u. Sohn, 1904, p. 51; Fischer. Vorlesungen über Bacterien. Jena: G. Fischer, 1903, p. 173.

and phosphin, while the mineral portion still remains combined with the residual organic material and is not directly available.[a] But whether decay or putrefaction predominates in the soil, the simplification of the nitrogenous organic substance involves the formation of ammonia, whose amount is determined by soil bacteriological activities.

Müntz and Coudon[b] demonstrated that no ammonification can take place in sterile soils and that the production of ammonia from nitrogenous organic substances is a property common to both molds and bacteria. The more systematic investigation of Marchal[c] showed that out of the 31 species tested 17 displayed a strong ammonifying power, prominent among them *Bacillus mycoides, Proteus vulgaris, B. mesentericus vulgatus, B. janthinus*, and *B. subtilis*. Most of the others showed a smaller, but none the less distinct, ammonifying power. Molds also proved themselves capable of splitting off ammonia from protein substances. A solution containing 1.365 grams of organic nitrogen per liter had 46 per cent of the nitrogen transformed into ammonia by *B. mycoides* within twenty days from the beginning of the experiment. An organism named by him N 1 transformed 39 per cent of it into ammonia; *Proteus vulgaris* transformed 36 per cent; *B. mesentericus vulgatus*, 29 per cent; *Sarcina lutea*, 27 per cent; *B. janthinus*, 23 per cent; *B. subtilis*, 19 per cent, and other organisms smaller quantities. Furthermore, of the eight cultures of *B. mycoides* derived from different sources, some showed a more pronounced ammonifying power than others. These observations, as well as others already reviewed, would indicate, therefore, that many of the organisms universally present in arable soils possess a strong ammonifying power, and that this power is variable even in the same species.

Marchal states that of the several organisms isolated from the soil, *B. mycoides* transforms albumin into ammonia most energetically; almost half of the organic nitrogen placed at its disposal was changed into ammonia. He also noted that when *B. mycoides* was inoculated into a neutral solution of albumin the medium became strongly alkaline after a short time, a phenomenon due to the accumulation of ammonium carbonate in the solution. Simultaneously with the appearance of ammonia there was a corresponding loss of albumin. The analysis of the atmosphere in which the culture was confined showed a marked absorption of oxygen and the formation of carbon dioxid. Hydrogen and nitro-

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[a] Wollny. Die Zersetzung der organischen Stoffe. Heidelberg: C. Winter, 1897, p. 8.

[b] Compt. Rend. Acad. Sci. [Paris], 116 (1893), p. 395; Ann. Agron., 19 (1893), p. 209.

[c] Bul. Acad. Roy. Sci. Belg., 3. ser., 25 (1893), p. 727.

gen were not present among the gaseous products of fermentation. The quantitative determination of the carbon dioxid and the ammonia formed in the respiration of this organism showed that the substances were nearly in proportion to the corresponding amounts formed in the complete combustion of albumin. Besides these two substances there were also found in the solution small quantities of the following compounds: Peptones, leucin, tyrosin, and formic, butyric, and propionic acids. In view of these facts Marchal believes himself justified in assuming that in the life processes of *B. mycoides* atmospheric oxygen is made to combine with the constituents of albumin, its carbon is transformed into carbon dioxid, the sulphur into sulphuric acid, a portion of the hydrogen into water, and ammonia seems to be a residual product. Marchal assumes the following equation:

$$C_{72}H_{112}N_{18}SO_{22}+77O_2=29H_2O+72CO_2+SO_3+18NH_3.$$

The intensity of the decomposition was sensibly influenced by the temperature, aeration, reaction, and concentration of the medium. It was found that only traces of ammonia were formed between 0° and 5° C.; at 10° the ammonia reaction was still slight, at 20° it was quite marked, and at 30° it attained its maximum. Being a distinctly aerobic organism (in the absence of nitrates), *B. mycoides* was found incapable of developing in a vacuum or in an atmosphere of hydrogen or carbon dioxid. The great avidity of this organism for oxygen was demonstrated by the proportionately better growth and the correspondingly increased production of ammonia with the increasing surface area of the culture solution. It was found further that *B. mycoides* could develop in a slightly acid medium, but grew most vigorously in a slightly alkaline medium.

Marchal concludes therefore that *B. mycoides* or "*Erde Bacillus*" of the German authors is one of the most common soil organisms and the one that attacks protein substances most energetically. The optimum conditions for its activity are: A temperature of about 30°, complete aeration, slightly alkaline medium, and a slight concentration of the nitrogenous substance in solution. It was found likewise that this organism can ammonify not only albumin, but also casein, fibrin, legumin, glutin, myosin, serin, and peptones. The same was true also of creatin, leucin, tyrosin, and asparagin; whereas urea, urea nitrate, and ammonium salts could not be used by this organism as sources of nitrogen.

It may be added here that our own experiments are confirmatory of the results secured by Marchal. Like the latter, we found *B. mycoides* capable of intense ammonification and of rapid degradation of protein bodies.

Marchal's results are amply confirmed by other investigations, including those of Severin,[a] Andrlik,[b] Schibata,[c] Chester,[d] Emmerling and Reiser,[e] Löhnis,[f] Gage,[g] and Lipman,[h] as well as the more general work of Berthelot.[i] It is sufficient to point out here, without going into too much detail, that Chester found all but one of the organisms tested by him, capable of producing ammonia under the conditions of the experiment, and that Gage noted the production of ammonia in thirteen out of the twenty cultures of sewage bacteria tested by him. The latter says:

> The tests for gelatin liquefaction were made on 157 of the before-mentioned cultures, and a comparison of the results of these tests with the various changes in the nitrogen content of the test solutions enables us to trace, in a general way, the relation between the peptonizing power as represented by the amount of liquefaction of gelatin and the denitrifying and ammonifying power. * * * Comparing the results obtained with cultures which liquefied with those which did not liquefy, we find that the liquefiers have an average ammonifying power nearly twice as great as the nonliquefiers.

These results are significant in that they demonstrate the greater ability of the gelatin-liquefying bacteria (that is, of those producing peptonizing ferments) to attack protein substances and to induce their decomposition with the formation of ammonia. Considering that the liquefying species may form at times more than 15 per cent[j] of the total number of soil bacteria which will form colonies on gelatin plates, it becomes apparent at once that these bacteria play an important rôle in the ammonification of soil nitrogen. It would seem that it is their function to do the initial work of rendering soluble the protein nitrogen in the soil humus, that it might be further decomposed by themselves or other soil organisms.

Turning to actual soil conditions, it will be recognized at once that the ammonification of soil nitrogen serves to regulate in a way the nitrogen feeding of crops. Soils whose ammonifying power is feeble can furnish but little nitrogen for further oxidation, and their production of nitrates must of necessity remain limited, even if we could

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[a] Centbl. Bakt. [etc.], 2. Abt., 13 (1904), p. 616.

[b] Ztschr. Zuckerindus. Böhmen, 27 (1902), p. 109; abs. in Centbl. Bakt. [etc.], 2. Abt., 10 (1903), p. 219.

[c] Beitr. Chem. Physiol. u. Path. [Hofmeister], 5 (1904), p. 384; abs. in Centbl. Bakt. [etc.], 2. Abt., 13 (1904), p. 230.

[d] Delaware Sta. Bul. 65.

[e] Ber. Deut. Chem. Gesell., 35 (1902), No. 1, p. 700.

[f] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 389.

[g] Jour. Amer. Chem. Soc., 27 (1905), No. 4, p. 327.

[h] New Jersey Stas. Rpt. 1906.

[i] Chimie Vegetale et Agricole. Paris: Masson et Cie., 1899, Vol. I, p. 160.

[j] Hiltner and Störmer. Studien über die Bakterienflora des Ackerbodens, etc. Berlin, 1903, p. 531. Reprinted from Arb. K. Gsndhtsamt., Biol. Abt., 3 (1903).

assume that they possess vigorous nitrifying bacteria. Those soils, on the other hand, which possess a strong ammonifying power are, other conditions being favorable, in a better position to yield an abundant supply of nitrates to the growing crop. These considerations led Remy[a] to study the ammonifying coefficients of a number of soils, as measured by their capacity to cause the production of ammonia in 1 per cent peptone solutions. Ehrenberg,[b] Wohltmann, Fischer and Schneider,[c] Löhnis,[d] and Chester,[e] were guided by the same considerations in their attempts to interpret ammonification as a function of soil fertility.

It should be remembered in this connection that by using equivalent quantities of the soils to be compared, for inoculation into a relatively large amount of culture fluid, we eliminate to a great extent the differences in mechanical or chemical constitution. The differences in ammonification in such solutions represent therefore only bacteriological differences. It does not follow, however, that the ammonifying or other soil bacteria would show proportionate differences in their natural habitat, since they are so essentially influenced by the aeration, moisture content, and reaction of the corresponding soil, as well as by its content of soluble salts, of organic matter, etc. Such soil dissimilarities may more than offset the bacteriological differences when tending in the opposite direction, or may further intensify them when tending in the same direction. It would seem more rational therefore to employ weighed quantities of the soils themselves, as was done, for instance, by King[f] in the examination of the water-soluble salts in soils, or by Withers and Fraps,[g] in the study of nitrification, were it not for the greater analytical difficulties, as well as the bacteriological complications introduced. Nevertheless, the two methods may be coordinated in a part of the bacteriological work at least. As it is, Remy's method alone has given an indication of future usefulness, as already noted (p. 12), and may on further study and modification prove even better adapted to aid us in the investigation of soil-fertility problems.

Among the experimental data secured in the laboratories of the New Jersey experiment stations there are some analyses showing the ammonifying power of certain clay-loam soils of the same origin, but

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[a] Centbl. Bakt. [etc.], 2. Abt., 8 (1902), p. 657; 5. Internat. Kong. Angew. Chem. [Berlin], 1903, Ber. 3, p. 793.

[b] Landw. Jahrb., 33 (1904), p. 131.

[c] Jour. Landw., 52 (1904), p. 97.

[d] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 389.

[e] Delaware Sta. Bul. 65, p. 51.

[f] Investigations in Soil Management. Madison, Wis.: Author, 1904.

[g] Jour. Amer. Chem. Soc., 24 (1902), No. 6, p. 528.

differentiated by manure and fertilizer treatment. The analytical returns show quite clearly a greater ammonifying power for the soils that have been receiving annual applications of manure, as compared with the soils which have received fertilizer. But what is of greater interest, these returns show also marked distinctions between soils treated with different fertilizers. It seems, moreover, that these differences, as brought out by Remy's method, show not merely bacteriological variations, but also chemical variations, since, as noted elsewhere (p. 12), the ammonifying power of any soil is but a function of the number and the physiological efficiency of its bacteria, as well as of its chemical constitution. Hence, we may look forward to future improvements in this method that will render it of practical value in the study of soil-fertility problems, and will permit us to distinguish soil differences, which we are at present unable to detect.

Recent work carried on under the direction of Remy at Bonn-Poppelsdorf furnishes still further support for this view. In the director's report [a] for 1905–6, Remy gives only the general conclusions, and claims that the still incomplete experimental data show strikingly that the bacteriological methods are well adapted to indicate rapidly and accurately the present fertility conditions of the soil; the bacteriological methods are superior in convenience and accuracy to other methods in this respect.

There is a special group of organisms capable of intensive ammonification. These are the so-called urea bacteria studied by Pasteur [b] and by many investigators after him. These organisms cause the hydration of urea:

$$\begin{matrix} NH_2 \\ CO \\ NH_2 \end{matrix} + 2H_2O = (NH_4)_2CO_3$$

by means of the enzym urase.[c] Their action is of special moment in the ammoniacal fermentation of urine, where extensive losses of nitrogen may occur in the volatilization of the ammonium carbonate. Their activities and those of other soil organisms have been made the subject of a very interesting study by Löhnis [d] in connection with the decomposition in the soil of the new artificial fertilizer calcium cyana-

[a] Ber. Tät. Inst. Bodenlehre u. Pflanzenbau, Bonn, 1906.

[b] Lafar. Technical Mycology, Vol. I, p. 332.

[c] Van Tieghem. Recherches sur la Fermentation de l'Urée. Thesis, Paris, P. Dupont, 1864. Musculus—Compt. Rend. Acad. Sci. [Paris], 82 (1876), p. 333. Miquel—Ann. Microg., 9 (1897), p. 302; cited after Duclaux, Traité de Microbiologie. Paris: Masson et Cie., 1899, vol. 2, p. 544. Beijerinck—Centbl. Bakt. [etc.], 2. Abt., 7 (1901), p. 33.

[d] Centbl. Bakt. [etc.], 2. Abt., 12 (1904), pp. 262, 448; 14 (1905), pp. 87, 389.

mid.[a] He finds in the transformation of calcium cyanamid a process somewhat analogous to that in the transformation of urea:

$$CaCN_2+H_2O=CNNH_2+CaO$$
$$CN.NH_2+H_2O=CO(NH_2)_2$$
$$CO(NH_2)_2+H_2O=2NH_3+CO_2$$

Löhnis has even introduced in his method of soil bacteriological study the use of urea solutions for the examination of the activities of the specific bacteria.[b]

OXIDATION PROCESSES IN THE SOIL.

The decomposition of organic matter in the soil is the outcome largely of oxidation processes. This is proved not merely by the fact that the oxygen of the organic material itself is insufficient for its complete oxidation, but likewise by the observations of Boussingault and Levy,[c] and the subsequent analyses of von Fodor,[d] that the soil

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[a] See Ashby, S. F. Jour. Agr. Sci., 1 (1905), p. 358.
Edler. Deut. Landw. Presse, 32 (1905), p. 5.
Erlwein, G. Electrochem. Ztschr., 28 (1907), pp. 41, 62.
Feilitzen, v. Verhandl. Gesell. Deut. Naturf. u. Aerzte [Breslau], 76 (1905), II, No. 1, p. 157; abs. in Centbl. Agr. Chem., 1906, p. 137.
Frank. 5. Internat. Kong. Angew. Chem. [Berlin], 1903, Ber. 3, p. 727; Ztschr. Angew. Chem., 19 (1906), p. 835.
Gerlach. Jahrb. Deut. Landw. Gesell., 19 (1904), p. 33.
Hall. Jour. Agr. Sci., 1 (1905), p. 146.
Hardt. Deut. Landw. Presse, 32 (1905), p. 827.
Haselhoff. Landw. Jahrb., 34 (1905), p. 597.
Immendorff. Fühling's Landw. Ztg., 54 (1905), p. 787.
Perotti. Staz. Sper. Agr. Ital., 37 (1904), p. 787; Arch. Farmacol. Sper. e Sci. Aff., 5 (1906), p. 385.
Remy, T. Landw. Jahrb., 35 (1906), Sup. 4, p. 114.
Rössler. Illus. Landw. Ztg., 25 (1905), p. 311.
Sebelien. Jour. Landw., 54 (1906), p. 159.
Seelhorst, v. Jour. Landw., 53 (1905), p. 338.
Söderbaum. Meddel. K. Landtbr. Akad. Exptlfält. [Stockholm], 1905, No. 85.
Strohmer. Mitt. Chem. Tech. Vers. Stat. Cent. Ver. Rübenz. Indus. Österr.-Ungar., 1905, No. 167.
Stutzer. Landw. Vers. Stat., 65 (1906), p. 275.
Tacke. Mitt. Ver. Förd. Moorkultur Deut. Reiche, 21 (1903), No. 23, p. 347.
Wagner. Deut. Landw. Presse, 30 (1903), p. 493.
Wein. Verhandl. Gesell. Deut. Naturf. u. Aerzte [Breslau], 76 (1905), II, No. 1, p. 162; abs. in Centbl. Agr. Chem., 1906, p. 137.
Zielstorff. Illus. Landw. Ztg., 1904, p. 1103.

[b] Centbl. Bakt. [etc.], 2. Abt., 12 (1904), p. 455.

[c] Compt. Rend. Acad. Sci. [Paris], 35 (1852), p. 765; abs. in Jahresber. Chem., 1852, p. 783.

[d] Deut. Vrtljschr. Öffentl. Gsndhtspflege., 7 (1875), p. 205.
Fodor, Josef. Hygienische Untersuchungen über Luft, Boden und Wasser. 2. Abt.: Boden und Wasser. Braunschweig: Friedrich Vieweg und Sohn, 1882, p. 28.
Wollny. Die Zersetzung der organischen Stoffe. Heidelberg: C. Winter, 1897, p. 118.

atmosphere becomes proportionately poorer in oxygen as it gains in its content of carbon dioxid, so that the total volume of the two gases is practically constant. For instance, it was found by Boussingault and Levy that a certain sandy soil that had been recently manured contained in its atmosphere 9.74 per cent of carbon dioxid, and 10.35 per cent of oxygen, or in all 20.09 per cent. Another sandy soil, taken from a vineyard, contained 1.06 per cent of carbon dioxid, and 19.72 per cent of oxygen, in all 20.78 per cent. Still a third sandy soil contained 0.87 per cent carbon dioxid and 19.61 per cent of oxygen, in all 20.48 per cent of the soil atmosphere. Von Fodor found on an average of 19 analyses 2.54 per cent of carbon dioxid and 18.33 per cent of oxygen, or a total volume of the two gases of 20.87 per cent in soil air; whereas the corresponding figures for atmospheric air were 0.04 per cent of carbon dioxid, 20.96 per cent of oxygen, a total volume of 21 per cent for the two gases. According to Wollny, these figures show clearly that the oxygen content of the soil air diminishes in the same degree as its content of carbon dioxid increases. Taken in conjunction with other facts, this would lead to the conclusion that atmospheric oxygen plays a controlling rôle in the oxidation of carbon.

The oxidation of the carbon and hydrogen involves also the formation of ammonia, as has been already shown in Marchal's studies with *Bacillus mycoides* (p. 50), and in general it may be said that the intensity of the oxidation processes is directly affected by the supply of oxygen. A neat demonstration of this was made by Wollny,[a] who passed air with a varying oxygen content through tubes containing a moist mixture of sand and powdered peat. The greatest amount of carbon dioxid was formed where pure oxygen was passed through the tubes, and the amount of the former rapidly diminished as the proportion of the oxygen in the air passed through the tubes was decreased. Similar observations were made by Schloesing,[b] who showed further that beyond a certain point the increase in the proportion of oxygen did not produce a corresponding increase in the amount of carbon dioxid formed.

It has already been observed in the discussion of Marchal's experiments (p. 50) that the formation of ammonia is favored by the unhindered access of oxygen, and that in this process considerable quantities of oxygen are used up and nearly corresponding quantities of carbon dioxid formed. We know, likewise, that the oxidation processes in the soil do not stop with the formation of ammonia. The latter is but a transition product in the transformation of soil nitrogen, and under normal conditions only small amounts of it are found in the soil at any time. Ammonia is itself the starting point in the work of

[a] Die Zersetzung der organischen Stoffe. Heidelberg: C. Winter, 1897, p. 117.

[b] Compt. Rend. Acad. Sci. [Paris], 77 (1873), pp. 203 353.

specific soil organisms, which complete the task of the great host of the decay bacteria proper. Nitrites and nitrates are formed, the last named being the final product in the oxidation of organic nitrogen broadly designated as nitrification.

NITRIFICATION.

It is to the arts of war, rather than to the arts of peace, that we owe much of our early knowledge of nitrification. With the increased use of gunpowder in warfare came an increased demand for saltpeter. It was this demand, and the inadequacy of the natural supply, that led to a more careful study of the natural niter beds and of the conditions most favorable for their formation. And, moreover, it led to the establishment of saltpeter plantations, or artificial niter beds. These, then, may be regarded as the first steps in the long-continued experimental study of a certain class of phenomena, that finally led to the modern theory of nitrification.[a]

The almost incessant wars of the seventeenth and eighteenth centuries constituted a most severe drain on the resources of the niter refineries. In many instances, the production of saltpeter came under government control, for in the great struggle then going on in Europe the different governments felt that a sufficient supply of niter was indispensable to their very existence. France, being cut off from the saltpeter beds of India and Bengal by the superior naval power of England, found herself compelled to tax her internal niter-producing capacity to the utmost. Laws were enacted compelling the peasants to furnish annually a certain amount of saltpeter as a part of their taxes, experts were appointed by the Government to superintend the preparation of artificial niter beds, and prizes were offered for the best methods of producing saltpeter. All this gave an impetus to the study of the question that was directly responsible for the accumulation of much valuable information, to be used later in the service of agriculture. Some very interesting work carried out recently by Müntz and Lainé[b] on the nitrification of peat nitrogen shows their method to be quite effective and much superior to the old methods employed on the saltpeter plantations.

Space will not allow here the consideration at length of the very extensive literature on nitrification. The earlier portion of it, dating back to the borderland between alchemy and chemistry, is strongly tinged with romance, and possesses a stirring interest for the modern agricultural chemist. He may see reflected in it the changing theories

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[a] Lipman, J. G. Studies in Nitrification. Master's Thesis, Cornell University, 1900.

[b] Compt. Rend. Acad. Sci. [Paris], 142 (1906), pp. 1239–1244; Ann. Inst. Agron., 2. ser., 6 (1907), No. 1, p. 15.

of contemporary science and the blind groping after truth. He may see, likewise, still another borderland, this time between chemistry and bacteriology, and the birth of agricultural bacteriology.

Lemery, who devoted himself to the study of nitrification, wrote at the beginning of the eighteenth century:[a]

> Saltpeter is to be regarded as a vegetable or animal salt, rather than a mineral salt; if the latter were true we should find saltpeter in the interior of the earth, in springs, etc. * * * All plants derive their juices and all their constituents from the earth, and animals subsist directly or indirectly on plants, and the saltpeter must have its origin in the soil.

About a half a century later there was published in Crell's "Die neuesten Entdeckungen in der Chemie" a review of some experiments carried out at the saltpeter works at Helsingförs. The review shows that but little advance had been made toward a rational theory of nitrification since Lemery's publication.

The importance of the problem led the Berlin Academy of Sciences to offer a prize for the best essay on the subject. The prize was awarded to a certain Pietsch, who tried to prove in the chemical language of the day that the new saltpeter has no perfect salty basis and that nitric acid consists of sulphuric acid and a fine fatty substance or combustible.

As to the then current belief regarding the nature of nitrates and nitric acid, the statement of Crell gives a fairly good notion. He says:

> Chemists differ as to the origin and essential constituents of nitric acid. Some regard it as a peculiar body floating in the air and following the north and west winds; some as a product of decay; some believe that it originates from common salt; and the majority and most up-to-date regard it as derived from carbonic acid gas (*Luftsäure*), which is a spirit of vitriol.

With the rapid advance of chemistry during the second half of the eighteenth and the beginning of the nineteenth centuries, the views on nitrification became more rational. As early as 1784 Cavendish had noticed that nitrate of potash is formed in a solution of potash when electric sparks are passed through moist air confined over this solution. This gave a clue to the development of a theory that the nitrates in the soil, or at least most of the nitrates in the soil, are derived from the oxidized nitrogen formed by electric discharges in the atmosphere. De Saussure stated[b] in 1809 that nitric and nitrous acid, as well as ammonia, are formed on the combustion of a mixture of oxygen and hydrogen in the air. For more than a half a century after that there prevailed among scientific men the exclusive belief in the purely chemical origin of nitrates, and occasional adherents to

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[a] Hist. Acad. Roy. Sci. * * * avec les Mémoires, 1717. Paris: Imprimérie Royale, 1719. Mém., p. 133. Cited from abs. in Crell's Neues Chem. Arch., 1 (1848), p. 169.

[b] Ann. Chem., 71 (1809), p. 254.

this view are met with well toward the end of the nineteenth century. The various chemical theories on nitrification are quite fully considered by S. W. Johnson[a] and need not be reviewed here. The great importance of the problem induced many prominent chemists and investigators to study the question. There had been much work on the subject, and still more discussion; still flaws were apparent in the best of the theories which attempted to prove that nitrification was a purely chemical process. The solution of the problem did not come until chemical and bacteriological methods had been considerably improved.

Pasteur, as early as 1862, suggested that nitrification was due to ferment action; and Müller observed that ammonia in sewage is rapidly converted into nitrates, while no corresponding change takes place in pure solutions of ammonia. These facts inclined him to the belief that nitrification is caused by a specific ferment. Experimental evidence on the subject was first furnished by Schloesing and Müntz,[b] who, in order to determine whether nitrification was really due to bacterial action, filled a glass tube with quartz sand, to which a little lime was admixed, and then passed sewage water through it. For twenty days the ammonia contained in the sewage passed through unchanged. At the end of that time nitrates appeared and increased in amount. The tube and contents were then treated with chloroform, and nitrification stopped. When a little fresh garden soil was added, nitrification again became active. These facts showed that a certain length of time was required for the bacteria to become active; that they succumbed to the vapors of chloroform, and that the soil itself was not so modified by the treatment as to be incapable of supporting further nitrification.

With the true nature of nitrification thus demonstrated, attempts were not wanting to secure pure cultures of nitrifying bacteria. One need but recall the patient work of Warington,[c] of Celli and Marino-Zuco,[d] and of the Franklands,[e] all of them doomed to failure because of the difficulty of the task before them. Winogradski,[f] who finally solved the problem, did so only after repeated discouragement, and it was left for him to explain the failure of the others. He demonstrated that the nitrifying bacteria do not grow on ordinary meat-extract gelatin, and that their successful isolation can be secured by

[a] How Crops Feed. New York: Orange Judd Co., 1870, pp. 70, 251.

[b] Compt. Rend. Acad. Sci. [Paris], 84 (1877), p. 301.

[c] Jour. Chem. Soc. [London], 59 (1891), p. 484.

[d] Naturw. Rundschau, 1 (1886), No. 41, p. 375; abs. in Centbl. Agr. Chem., 16 (1887), p. 292.

[e] Proc. Roy. Soc. London, 47 (1890), p. 296; abs. in Jour. Chem. Soc. [London], 60 (1891), p. 352.

[f] Ann. Inst. Pasteur, 4 (1890), pp. 213, 257; abs. in Centbl. Agr. Chem., 19 (1890), p. 641.

means of purely inorganic media. (It was subsequently shown that organic media freed from soluble material by careful washing may also be used for the purpose.) The isolation in pure culture of the nitrous and nitric ferments forms one of the proudest achievements in the realm of agricultural bacteriology. It has been productive of a constantly growing interest in soil biological problems, and has undoubtedly served as a stimulus for much valuable investigation.

The laboratory studies served to explain not a little that had hitherto been obscure in field practice. Thus Schloesing's[a] demonstration that the nitrates formed are in direct proportion to the amount of oxygen supplied; Davy's[b] observation that nitrification is greatly modified by temperature and that an excess of animal matter in solution retards nitrification; Reder's[c] proof that nitrification is aided by lime, lime carbonate, magnesia, and especially potassium carbonate, and Pichard's[d] conclusion that the sulphates of calcium, potassium, and sodium promote nitrification were not devoid of considerable practical significance. The same may be said of a vast amount of similar research by Warington,[e] Dehérain,[f] Pichard,[g] Leone,[h] Dumont and Crochetelle,[i] Dehérain and Pagnoul,[j] Pagnoul,[k] Marcille,[l] Godlewski,[m] Paturel,[n] Kochenovski,[o] Hartleb,[p] the younger Schloesing,[q] Migula,[r] King,[s] and a host of others. In field practice

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[a] Compt. Rend. Acad. Sci. [Paris], 77 (1873), pp. 203, 353.

[b] Chem. News, 40 (1879), p. 271.

[c] Wchnschr. Pommer. Ökonom. Gesell., 1884, No. 17, p. 114; abs. in Centbl. Agr. Chem., 13 (1884), p. 652.

[d] Compt. Rend. Acad. Sci. [Paris], 98 (1884), p. 1289.

[e] Ann. Agron., 11 (1885), p. 557.

[f] Ann. Agron., 13 (1887), pp. 241–261; 19 (1893), p. 401; Jour. Agr. Prat., 57 (1893), I, p. 742.

[g] Jour. Agr. Prat., 53 (1889), II, pp. 373, 374; Ann. Agron., 18 (1892), pp. 108–119; Compt. Rend. Acad. Sci. [Paris], 114 (1892), p. 490.

[h] Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., 4. ser., 6 (1890), I, p. 33; abs. in Naturw. Rundschau, 5 (1890), p. 291.

[i] Compt. Rend. Acad. Sci. [Paris], 117 (1893), p. 670; 119 (1894), p. 93.

[j] Ann. Agron., 20 (1894), p. 449; 21 (1895), pp. 193, 207.

[k] Compt. Rend. Acad. Sci. [Paris], 120 (1895), p. 812; Ann. Sci. Agron., 2. ser., 4 (1898), II, No. 1, p. 97.

[l] Ann. Agron., 22 (1896), p. 337.

[m] Bul. Internat. Acad. Sci. Cracovie, Compt. Rend., 1892, p. 408; abs. in Forsch. Agr. Phys. [Wollny], 16 (1893), p. 240; Centbl. Bakt. [etc.], 2. Abt., 13 (1893), p. 559.

[n] Ann. Agron., 22 (1896), p. 511.

[o] Selsk. Khoz. i Lyesov., 182 (1896), p. 455; abs. in Expt. Sta. Rec., 8, p. 871.

[p] Stutzer and Hartleb. Centbl. Bakt. [etc.], 2. Abt., 2 (1896), p. 701; 3 (1897), pp. 55, 161, 235, 311, 351.

[q] Compt. Rend. Acad. Sci. [Paris], 125 (1897), p. 824.

[r] Centbl. Bakt. [etc.], 2. Abt., 6 (1900), p. 365.

[s] See King and Jeffrey. Wisconsin Sta. Rpt. 1899, p. 219.
King and Whitson. Wisconsin Sta. Rpt. 1900, p. 204; 1901, p. 210.
Whitson, Wells, and Vivian. Wisconsin Sta. Rpt. 1902, p. 192.

the investigations of the Rothamsted Station have made a generous contribution to our knowledge of nitrification. The extent and rapidity with which ammonia and ammonium salts are nitrified had already been indicated by numerous laboratory experiments,[a] but the results obtained on the Rothamsted fields are sufficient to convince the most skeptical. Concerning these results Dyer says:[b]

It is observed at Rothamsted, when ammonium salts are applied, say, at the end of October, that if the drainpipes happen to run within a week or two of the sowing, the first collection of the drainage water will show traces of ammonia; but that, if there be only a day or two of continuous drainage, even so late in the year, the ammonia often wholly disappears, the whole of the nitrogen in the drainage being already converted into nitrates. Indeed, in the earlier years of the experiments, when the ammonium salts were throughout for the most part applied in autumn, the quantity of nitric nitrogen in the winter drainage of the different plats obviously bore a very direct relation to the quantities of ammonium salts applied.

The application of ammonium salts is, in fact, virtually tantamount to an application of nitrate, and it is only because ammonium salts are capable of such rapid conversion into nitrate that they give, in practical farming, under favorable conditions of soil and weather, results closely approximating those obtained by the application of sodium nitrate itself. * * *

Nitrification goes on mainly, though probably not exclusively, in the surface soil or in the upper depths of the subsoil. But even if the actual conditions naturally existing in the subsoil allow of any appreciable nitrification in the lower depths, it would seem to be very feeble. * * *

On the whole, then, we may consider that the vast stores of subsoil nitrogen within the reach even of the deeply rooting wheat plant are, for all practical or economical purposes, unavailable for appreciable contribution to the nitrogenous sustenance of the crop.

Even of greater interest is Dyer's account of the nitrogen balance in the soil where annual applications of manure were made for fifty years. It gives us an insight into the transformations and migrations of nitrogen in the soil and helps us to understand the relations in the decomposition of nitrogenous organic substance, the formation of ammonia, the oxidation of this to nitrates, the wanderings of the soluble nitrates, and the formation of gaseous nitrogen in the course of the various transformations. The balance sheet shows the following:

The manured plat has yielded in its crops in fifty years something like 1,600 pounds of nitrogen per acre more than the unmanured plat. Of the total 10,000 pounds of nitrogen estimated to have been supplied, then, we find (in rough round numbers) that 1,600 pounds have been recovered in the increased crops and that about 2,500 pounds are found in the surface soil, leaving 5,900 (or, in round numbers, 6,000) pounds to be accounted for otherwise. * * * It is clear, therefore, that, in spite of the notable surface accumulation, but little of the large quantities of nitrogen supplied in the dung and not returned in crops is to be found in the subsoil. The greater part of it has disappeared, either as nitrates in the drainage or perhaps, and probably largely, by fermentative processes yielding free nitrogen.[c]

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[a] Wollny. Die Zersetzung der organischen Stoffe. Heidelberg: C. Winter, 1897, p. 3.
[b] U. S. Dept. Agr., Office Expt. Stas. Bul. 106, pp. 64, 65, 67, 71.
[c] Ibid., pp. 38, 99.

That such enormous losses are not exceptional and are due, in part at least, to excessive oxidation and the leaching away of the nitrates formed is corroborated by Snyder,[a] who found in the continuous cultivation of wheat a loss of 2,039 pounds of nitrogen per acre in twelve years. During that time the crops removed less than 450 pounds, the rest having been lost. "For every pound of nitrogen removed there has been a loss of over 4 pounds from the soil by the decay of the humus."

The rapidity with which organic or ammoniacal nitrogen may be nitrified in the soil depends largely on the various factors already referred to in this paper. These factors of temperature, moisture, aeration, salt content, etc., affect also the other bacteriological processes. In the case of nitrification, particularly, extensive study has been made of the comparative rates of transformation for different nitrogenous materials, and tables have been prepared to show the comparative manurial value of these materials.[b] It has been demonstrated, for instance, that green-manure nitrogen has a much higher availability than stable-manure nitrogen, and this in turn shows a higher availability than leather-meal nitrogen. The experiments of one of the writers have demonstrated how great the variations may be, even in manure samples of different composition. Thus it was shown that fresh solid manure had an availability of 43.1 per cent in 1899, as against an availability of 88.4 per cent in the same year for fresh, solid, and liquid manure, the corresponding figures for 1900 being 26.4 and 51.5 per cent, respectively.[c] Both seasonal and constitutional influences are apparent in these differences.

It should be remembered in this connection that such availability studies deal not alone with the oxidation of ammonia to nitrates, but with the entire process of converting organic protein nitrogen into the inorganic nitrogen of nitrates. The term nitrification is frequently used in the broader sense, and includes then, also, the complex work of a host of soil organisms, the algebraic sum of whose physiological activities we have attempted to designate as ammonification. Under certain conditions ammonification may proceed normally, while nitrification proper is retarded, as has been pointed out by Pagnoul.[d] Space forbids the discussion here of Winogradski's more recent contributions to the physiology of nitrification,[e] of Ome-

[a] Minnesota Sta. Bul. 89, p. 193.

[b] Wagner and Dorsch. Die Stickstoffdüngung der landwirtschaftlichen Kulturpflanzen. Berlin: Paul Parey, 1892.

[c] Voorhees, E. B. Investigations Relative to the Use of Nitrogenous Materials. New Jersey Stas. Rpt. 1903, p. 172.

[d] Compt. Rend. Acad. Sci. [Paris], 120 (1895), p. 812.

[e] Centbl. Bakt. [etc.], 2. Abt., 2 (1896), pp. 415, 449. Winogradski and Omelianski. Centbl. Bakt. [etc.], 2. Abt., 5 (1899), pp. 329, 377, 429.

lianski's studies,[a] and of the quite recent critical discussion of some of Winogradski's conclusions by Löhnis.[b] But taking nitrification in its broader sense, and considering its more practical side, there is one phase of it deserving more than passing notice. It concerns the nitrifying power of different soils, and the variable amounts of nitrate formed in them under different conditions of season and treatment. Distinction is made here of the inherent differences in the soils themselves—in other words, in the qualitative and quantitative differences of the nitrifying organisms in the corresponding soils. The systematic study of soil nitrification is thus attempted as an aid in the measurement of soil fertility.

Marcille[c] compared the nitrifying power of three different soils, and found that the poorest yielded an organism nitrifying less rapidly than the others. A more systematic study in this direction was made by Jensen,[d] and more recently by Withers and Fraps.[e] The latter found that some soils nitrified ammonia nitrogen more readily, while others nitrified cotton-seed meal more rapidly. They state that "the addition of calcium carbonate invariably caused increased nitrification, if any nitrification at all took place. * * * In some soils nitrification did not occur when calcium carbonate was not added. Previous liming of the soil did not keep the calcium carbonate from being effective."

King[f] also showed the variable nitrifying power of different soils. Comparing four poorer soils with four stronger soils, he found that while the former produced on the average 15.20 parts of nitric acid per million of dry soil, the latter produced 43.79 parts per million. When manure at the rate of 5, 10, and 15 tons per acre was added, the effect on the poorer and stronger soils was dissimilar.

> From this comparison it appears that the addition of manure to the four poorer soils has augmented the development of nitrates and in amounts increasing with the manure added. * * * In the case of the four stronger soils, the two larger amounts of manure added appear to have retarded the accumulation of nitrates in the soil. The two groups of soils, therefore, hold opposite relations as regards the influence the manure has had upon their nitrate content. Such relations as these have been many times noted by different observers, and it is unfortunate that it has not yet been clearly demonstrated to what causes such relations should be ascribed.

It now remains to mention the results that have been obtained by Remy and by others who followed his method as indicated in the

[a] Centbl. Bakt. [etc.], 2. Abt., 5 (1899), p. 537; 8 (1902), pp. 193, 225, 257, 289, 321, 353, 385, 605, 785; 9 (1902), pp. 63, 113.

[b] Centbl. Bakt. [etc.], 2. Abt., 13 (1904), p. 706.

[c] Ann. Agron., 22 (1896), p. 337.

[d] Salpeterbakteriernes Utbredelse i Danmark, Tidsskr. Landbr. Planteavl. 5 (1899), p. 173; cited after Ehrenberg, Landw. Jahrb., 33 (1904), p. 8.

[e] Jour. Amer. Chem. Soc., 24 (1902), No. 6, p. 528; 5. Internat. Kong. Angew. Chem. [Berlin], 1903, Ber. 3, p. 926.

[f] Investigation in Soil Management. Madison, Wis.: Author. 1904. p. 30.

preceding pages. Remy himself proved that there is a direct relation between the nitrifying power of a soil and the availability of the ammonium salts applied to it,[a] although the relations are by no means constant. Uncertain results were obtained by Ehrenberg,[b] yet he is led to conclude that there is a relation between nitrification in nutrient solutions and in the soil. Wohltmann, Fischer, and Schneider obtained more encouraging results,[c] and believe that the investigations on the influence of different fertilizers or combinations of fertilizers on the nitrifying power of the soil have demonstrated its intimate relation to the chemical composition of the soil. It should be added here, however, that their investigations dealt with soils originally identical, but differentiated by methods of treatment, and it is questionable whether coordinate results could have been secured as easily on soils of different geological origin.

As far as the nitrogen feeding of plants is concerned, the oxidation of ammonia to nitrates is not as absolutely indispensable as is frequently supposed. The investigations of Müntz,[d] Laurent,[e] and Griffiths[f] showed that in sterilized soils ammonium salts are assimilated by plants. Gerlach and Vogel[g] show in their experiments with Indian corn that in soils kept sterile no nitrification of ammonium salts occurred, and no nitrous or nitric ferments could be detected either during the experiment or at the end of it. Nevertheless, the corn plants grew normally where ammonium salts were supplied and showed a considerable gain of both dry matter and of nitrogen over the corresponding plants in the soils where no ammonium salts were applied. They conclude, therefore, that plants can make use of ammonia in ammonium sulphate for the building of protein bodies. The more recent work of Krüger[h] carried on with various plants also shows that ammonium salts may be used directly as a source of nitrogen, and that different crops show notable differences in their attitude toward the various compounds of nitrogen, which they use for their life processes. Krüger summarizes the results secured by him in 1899, 1903, and 1904 as follows: (1) Mustard, oats, and barley seem to show no preference for either ammonia or nitrate nitrogen, and utilize the two impartially for the building of plant substance; (2) potatoes seem to prefer ammonia nitrogen to nitrate nitrogen, although the action of the latter is by no means inferior to that of the former; (3) beets have a decided preference for nitric nitrogen,

[a] 5. Internat. Kong. Angew. Chem. [Berlin], 1903, Ber. 3, p. 793.

[b] Landw. Jahrb., 33 (1904), p. 49.

[c] Jour. Landw., 52 (1904), p. 119.

[d] Compt. Rend. Acad. Sci. [Paris], 109 (1889), p. 646.

[e] Ann. Inst. Pasteur, 3 (1889), p. 362.

[f] Chem. News, 64 (1891), p. 147.

[g] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 124.

[h] Landw. Jahrb., 34 (1905), No. 5, p. 761.

and utilize it to much better advantage than corresponding amounts of ammonia nitrogen, the development of their root system seeming to be particularly favored by nitrate nitrogen; (4) the fact that the returns from ammonia nitrogen have been found, in practice, to be inferior to those from nitrate nitrogen is accounted for largely by micro-organic processes in the soil, rather than by the unequal physiological effect of the two salts; (5) cultivated plants, therefore, not only possess the power to utilize ammonia as a source of nitrogen, but they are also able to a greater or less extent to utilize it in the same degree as nitrate nitrogen. He concludes that nitrification is, therefore, by no means as necessary for our cultivated plants as is commonly supposed, but that the methods employed in practical agriculture to facilitate the supply of soil nitrogen to crops by tillage, etc., are not in any way affected by this conclusion, since methods of soil treatment favorably affecting nitrification also favor the solution of the inert soil nitrogen.

The above experiments of Krüger are quite instructive, and valuable in so far as they add something to a clear understanding of the biological processes in the soil. From the standpoint of practical agriculture their value is not so great, for we know from numerous experiments and from the convincing data secured in the Rothamsted fields that ammonia nitrogen is quite rapidly nitrified in soils of normal constitution, and that, therefore, in the feeding of cultivated plants we must reckon with the processes of nitrification, and with the various conditions of climate, soil, and treatment that in any way affect these processes.

It may not be out of place, also, to say a word here in regard to calcium cyanamid and the crude (basic) calcium nitrate prepared by the Birkeland-Eyde process, which are coming into prominence in Europe as sources of nitrogen for crops and will probably find still more extensive use in agriculture, since in the use of these substances certain effects are produced on the bacterial flora of the soil that merit our attention.

Sebelien [a] calls attention to the irregular returns secured from the use of calcium cyanamid by a large number of investigators. This irregular action is unquestionably affected by the chemical and physical properties of the different soils, and quite as certainly by their biological character. With the exception of the work of Löhnis, [b] there is scarcely anything to add to our information on the subject. It appears, however, that as a rule the best results are secured from the use of calcium cyanamid only when a sufficient interval is allowed to elapse between its application and the planting

[a] Jour. Landw., 54 (1906), No. 2, p. 159.

[b] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), pp. 87, 389.

of the crop. There is scarcely any doubt that it must undergo certain modifications before it becomes acceptable to the higher plants, and these modifications bear an intimate relation to the life processes of certain species of soil bacteria. Sebelien also observed that calcium cyanamid is uncertain in its action, and inferior to sodium nitrate as a source of nitrogen; whereas the basic calcium nitrate proved in most of his experiments superior to the latter. This superior action of calcium nitrate was found to be due largely to the large quantities of lime admixed with it, since the yields from the sodium nitrate were also increased by the addition of lime carbonate. Nevertheless, even the lime did not in all cases increase the yield from the sodium nitrate to make the latter equal to that from the calcium nitrate. On the other hand, in the case of oats on a clayey soil the sodium nitrate gave larger returns than the calcium nitrate, and when lime was added to the sodium nitrate the yield from the latter was depressed. We do not know, of course,whether these peculiar results were due to purely physiological causes or whether they were affected by bacteriological activities. Certain it is that similar peculiarities have been observed time and again in actual field practice, and we may hope that time will bring a clearer understanding of these processes. Meanwhile we must remember that the nitrogen salts applied to the soil serve as food to the great host of soil bacteria, as well as to the higher plants; and hence the different effects produced on the soil bacteria by such substances as ammonium sulphate, calcium cyanamid, calcium nitrate, and sodium nitrate must necessarily be reflected in the growth of the crops.

As far as the action of the lime itself is concerned the prevailing uncertainty is quite marked. It is well understood that under certain conditions its influence may concern, above all things, the mechanical properties of the soil, and that its effect under other conditions may be predominatingly chemical, physiological, or bacteriological. At any rate, there is much that is suggestive in the work of Loew [a] on the effect of potassium oxalate and of other lime precipitants on cell nuclei. Loew concluded from his investigations that the calcium of the cell nuclei exists in combination with the nucleo-proteids (plastin and chromatin), and that when it is precipitated out as oxalate and is replaced by other bases the resulting changes that occur in the cell nuclei lead to their death. To confirm this view he tried other precipitants of lime, such as sodium fluorid, and later potassium carbonate, and secured like results. On the other hand, monopotassium and dipotassium phosphates failed to produce the same effect. They do not precipitate the calcium in the nuclei, probably because it already exists there in combination with phosphorus. Loew thinks that the poisonous action of lime pre-

[a] Bul. Col. Agr., Tokyo Imp. Univ., 7 (1906), p. 7.

cipitants is in some way intimately connected with the injurious action of magnesium salts on higher plants, which may be counteracted by the addition of lime. The lower algæ and molds, which can live without lime and are not therefore poisoned by oxalic acid, are not affected injuriously by an excess of lime. In view of the fact that *Aspergillus niger* and other molds are capable of producing oxalic acid from various substances, and particularly from amino acids, peptones, and protein bodies, as was demonstrated by Wehmer,[a] Emmerling,[b] and Heinze,[c] and, furthermore, that these molds develop well in acid soil, and especially those manured with ammonium salts, there is reason to think that the beneficial action of lime under such conditions may possibly have something to do with the facts pointed out by Loew. Theoretically at least there is good reason for supposing that the production of considerable quantities of oxalic acid or of ammonium oxalate in the soil would lead to physiological disturbances noted by Loew in the case of Spirogira. This would further serve to explain, perhaps, why some crops are more resistant to soil acidity than others, for it could be assumed here that the cell nuclei of some plants do not part as readily with their lime as do the cell nuclei of others, or that the lime requirements of some are quantitatively different from those of others. At best very little is known of the physiological and bacteriological effects that lime, or the absence of lime, may produce. We can only hope that here too more knowledge will be given us in the course of time. For one who has the training and the inclination this should prove a grateful field for inquiry, with many facts in store that will prove of deep interest to agricultural science.

DEOXIDATION PROCESSES IN THE SOIL.

With an insufficient supply of air the decomposition of organic matter in the soil may partake of the nature of putrefaction rather than that of decay proper. The great demand for oxygen under such conditions may lead to its withdrawal from organic, as well as inorganic, compounds. Attention has already been called to the partial deoxidation of the higher oxids of iron and manganese. The reduction of carbonate is not excluded, and the interesting work of Van Delden[d] with pure cultures of *Microspira desulphuricans* and *M. æstuarii,* both sulphate-reducing organisms, gives an insight into certain bacterial processes. With such organic compounds as sodium lactate or potassium malate as the sole source of energy he accomplished the reduction of magnesium sulphate to hydrogen sul-

[a] Bot. Ztg., 49 (1891), p. 233.
[b] Centbl. Bakt. [etc.], 2. Abt., 10 (1903), p. 273.
[c] Ibid., 12 (1904), p. 180.
[d] Ibid., 11 (1904), pp. 81, 113.

phid, carbon dioxid being another of the gaseous products formed. He has expressed the belief that his experiments have demonstrated that the reduction of sulphates by *M. desulfuricans* and *M. æstuarii* is a reaction accomplished only under anaerobic conditions and in a medium which contains besides sulphates also some suitable organic food. Although dealing only with such easily oxidizable substances as lactates or malates, the experiments proved to his satisfaction that a whole series of organic compounds may be oxidized by means of the sulphate oxygen, so that this oxygen acts in the self-purification of water and in the biological purification of sewage in a manner analogous to that of nitrate nitrogen. Denitrification, which was studied in greater detail in this connection by Van Iterson, finds, therefore, a biological analogy in sulphate reduction.

It thus appears that various reduction processes may prevail in the soil under certain conditions. Among these reduction processes that of the reduction of nitrates, commonly designated as denitrification, has been the subject of considerable study on the part of the agricultural and sanitary bacteriologist.

DENITRIFICATION.

The study of denitrification has not as long nor as varied a history as that of nitrification. Earlier observers who took cognizance of the reduction of nitrates were willing to regard it as a purely chemical process; in fact, contemporary knowledge allowed no other interpretation. Kuhlmann,[a] as far back as 1846, expressed the belief that nitric acid may be reduced in the soil to ammonia by the fermentation of organic substances. Froehde in 1867[b] and Angus Smith[c] in the same year, noted the reduction of nitrates in the presence of organic substances, a fact quite widely known toward the beginning of the seventies of the last century. The views of that time are well reflected in the following statement by Johnson:[d]

> In one and the same soil the conditions may exist at different times that favor nitrification on the one hand and reduction of nitrates to ammonia on the other. A surplus of moisture might so exclude air from a porous soil as to cause reduction to take place, to be succeeded by rapid nitrification as the soil becomes more dry.
>
> It is possible that nitrates may undergo further chemical alteration in the presence of excess of organic matters. That nitrites may often exist in the soil is evident from what has been written with regard to the mutual convertibility of nitrates and nitrites. According to Goppelsrörer, certain soils rich in humus possess in a high

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[a] Compt. Rend. Acad. Sci. [Paris], 23 (1846), p. 1033; Ann. Chim. et Phys., 3. ser., 20 (1847), p. 223; Jour. Prakt. Chem., 41 (1847), p. 289.

[b] Jour. Prakt. Chem., 102 (1867), p. 46.

[c] Cited after Aikman, Manures and the Principles of Manuring. Edinburgh and London: Wm. Blackwood and Sons, 1894, p. 177.

[d] How Crops Feed. New York: Orange Judd Co., 1870, p. 269.

degree the power to reduce nitrates to nitrites. It is not unlikely that further reduction may occur—that, in fact, the deoxidation may be complete and free nitrogen be disengaged. This is a question eminently worthy of study.

When Johnson wrote these words, the ground was already prepared for the recognition of biological factors in the reduction of nitrates. Schönbein[a] pointed out in 1868 that nitrates may be reduced to nitrites by fungi and that the presence of nitrites in drinking water may indicate that it contains micro-organisms. The same view as to the presence of nitrites in drinking water was also held by Meusel[b] and the idea was gradually gaining ground that the reduction of nitrates is in some way connected with bacteriological activities. With the beginning of the eighties, this view was substantiated by experimental research.

Dehérain and Maquenne[c] attributed the reduction of nitrates to anaerobic ferments and showed that the gases evolved contained, besides carbon dioxid, also free nitrogen and nitrous oxid. It remained for Gayon and Dupetit,[d] however, to demonstrate unequivocally the true character of denitrification. They described an anaerobic "ferment" capable of reducing nitrates rapidly. In this process gaseous nitrogen was set free. They also found that the ferments needed organic matter for their development. Observations of a like nature were made by Heraeus[e] and Springer.[f] In 1886 Gayon and Dupetit isolated[g] two organisms, *Bacterium denitrificans* α and *B. denitrificans* β, capable of reducing nitrates with the evolution of gaseous nitrogen. Besides these, they encountered a number of bacteria that could reduce nitrates to nitrites. Even more definite than Gayon and Dupetit's work were the studies of Giltay and Aberson,[h] who found a bacillus that could reduce considerable quantities of nitrates in nitrate bouillon and artificial solutions with the evolution of practically the entire amount of nitrogen in the elementary state.

All these investigations, while interesting in themselves, received an added interest with Bréal's[i] announcement that many substances of organic origin, and especially straw, are the carriers of denitrifying organisms. This announcement, together with the subsequent dec-

[a] Jour. Prakt. Chem., 105 (1868), p. 211.

[b] Ann. Chim. et Phys., 5. ser., 7 (1876), p. 287.

[c] Ann. Agron., 9 (1883), p. 6.

[d] Compt. Rend. Acad. Sci. [Paris], 95 (1882), pp. 644, 1365.

[e] Ztschr. Hyg., 1 (1886), p. 193.

[f] Amer. Chem. Jour., 4 (1883), p. 452; abs. in Ber. Deut. Chem. Gesell., 16 (1883), I, p. 1228.

[g] Rechercehs sur la reduction des nitrates pas les infiniment petits. Bordeaux: G. Gounouilhou, 1886; Mem. Soc. Sci. Phys. et Nat. Bordeaux, 3. ser., 2 (1886), p. 202.

[h] Arch. Neerland. Sci. Exact. et Nat., 25 (1891), pp. 341–361; cited after Burri and Stutzer, Centbl. Bakt. [etc.], 2. Abt., 1 (1895), p. 261.

[i] Ann. Agron., 18 (1892), p. 181.

laration of Wagner,[a] that denitrification may take place extensively in cultivated soils, lent the subject a great practical significance. According to him, applications of cow or horse manure to the soil are often not only unprofitable but harmful, since, when applied together with nitrates, they cause, by virtue of the micro-organisms contained in them, the destruction of the nitrates. More than that, the harmful effects do not stop here, for the nitrates, as they are gradually formed from the organic matter of the soil, are also attacked by the denitrifying bacteria and their nitrogen is set free.

Wagner's strong statement has been largely responsible for the very considerable amount of work on denitrification accomplished within the last ten years. Burri and Stutzer[b] isolated in pure culture two denitrifying organisms, one capable of completely reducing nitrates when growing alone, the other capable of this only when growing together with *Bacterium coli* or other members of the coli group. Their description, unlike that of Gayon and Dupetit, is sufficiently clear to allow the recognition of the two denitrifying organisms for purposes of isolation. In communicating the results of their experiments they state that there are but a limited number of organisms capable of oxidizing nitrogen, while of those possessing the power of deoxidation (reduction) the number is great. Of these, however, the greater part can only reduce nitrates to nitrites, and the bacteria capable of reducing nitrates to ammonia, or of setting nitrogen free, are not very numerous. Since the publication of these results the number of denitrifying organisms isolated, that is, of those capable of completely deoxidizing nitrates with the evolution of gaseous nitrogen, has become very considerable, and the statement of Burri and Stutzer is, therefore, only comparatively true. At any rate, the laboratory studies of Schirokikh,[c] Stutzer and Maul,[d] Garino and Ampola,[e] Severin,[f] Hjalmar Jensen,[g] Maassen,[h] Wolf,[i] Hoflich,[j] Christensen,[k] Van Iterson,[l] Stoklasa and Vitek,[m] of one of the writers,[n] and of many

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[a] Deut. Landw. Presse, 22 (1895), p. 83.

[b] Centbl. Bakt. [etc.], 2. Abt., 1 (1895), pp. 257, 350, 392, 422.

[c] Ibid., 2 (1896), p. 204.

[d] Ibid., p. 473.

[e] Ibid., p. 670.

[f] Ibid., 3 (1897), pp. 504, 554.

[g] Ibid., pp. 622, 689.

[h] Arb. K. Gsndhtsamt., 18 (1901), No. 1, p. 21; abs. in Centbl. Bakt. [etc.], 2. Abt., 8 (1902), p. 151.

[i] Hyg. Rundschau, 9 (1899), No. 11, p. 538; abs. in Centbl. Bakt. [etc.], 2. Abt., 5 (1899), p. 682.

[j] Centbl. Bakt. [etc.], 2. Abt., 8 (1902), pp. 245, 273, 305, 336, 361, 398.

[k] Ibid., 11 (1903), p. 190.

[l] Ibid., 12 (1904), p. 106.

[m] Ztschr. Landw. Versuchw. Österr., 9 (1906), pp. 49, 844.

[n] New Jersey Stas. Rpt. 1902, p. 183.

others show clearly enough that the number of organisms capable of reducing nitrates partially or wholly is great indeed.

Yet whatever the interest attached to the laboratory studies of pure cultures of denitrifying bacteria, it was necessary to determine once for all the exact relation of denitrification processes to crop production; in other words, to test the theories by vegetation experiments and by actual field practice. The very interesting work along these lines by the German experiment stations and by individual investigators is reviewed elsewhere.[a] It is sufficient to state in this place that by means of numerous pot and field experiments, including the extensive cylinder experiments at the New Jersey Station,[b] it has been amply demonstrated that there need be no fear of extensive nitrogen losses in practical agriculture from the complete reduction of nitrates. Where such losses do occasionally occur they follow the excessive applications of organic manures, or other soil conditions that may justly be designated as abnormal.

There is, however, a series of bacteriological processes in the soil which are frequently, but improperly, designated by the term denitrification, and these processes are undoubtedly of no small significance in the economy of soil nitrogen. The term denitrification in its proper and more limited sense refers only to the complete reduction of nitrates with the evolution of elementary nitrogen. It is also applied in a broader way to all deoxidation processes whereby nitrates are partly or wholly reduced. In this respect there is analogous confusion in the definition of nitrification and denitrification, either or both being used in a broader or narrower sense. But, whereas the term nitrification is always conditioned upon the end products formed, whether the oxidation begins at the organic, ammonia, or nitrite stage, the term "denitrification" is definite only when used in the narrower sense. For practical agriculture, however, the differences are of some moment. The partial reduction of nitrates to nitrites or to ammonia does not necessarily involve a loss of soil nitrogen; but the complete reduction of nitrates, wherever it occurs, must of necessity involve such losses. As it is, there is some justification, at least, for referring to the partial reduction of nitrates as denitrification. When, however, an attempt is made to classify under the head of denitrification all bacterial activities in the soil that lead to the disappearance of the nitrate reaction, or even to the diminution in the total store of soil nitrogen, it can hardly be regarded as justifiable. It has been repeatedly demonstrated[c] that the nitrate reaction in the culture solutions

[a] New Jersey Stas. Rpt. 1901, p. 183.

[b] New Jersey Stas. Rpts. 1899, p. 97; 1900, p. 88; 1901, p. 144; 1902, p. 133; 1903, p. 148; 1904, p. 191.

[c] Lemmermann. Kritische Studien über Denitrifikationsvorgänge. [Habilitationsschrift.] Jena, 1900, p. 25.

may completely disappear without involving any loss of nitrogen. Gerlach and Vogel[a] showed that certain bacteria in the soil may transform nitrate nitrogen into protein nitrogen almost quantitatively, and more recently Löhnis[b] confirmed their results. The nitrogen thus rendered insoluble is by no means lost and may again become available through decomposition and oxidation processes. Evidently it would be incorrect to designate such temporary withdrawal of the soluble nitrate nitrogen from circulation as denitrification. Yet this mistake is frequently made when qualitative tests are alone depended upon. Furthermore, losses of nitrogen may occur in various oxidation processes,[c] as has been mentioned under the head of ammonification. Free nitrogen, ammonia, or ammonia derivatives may thus be evolved and lost, and where the process is imperfectly understood these losses may be attributed to denitrification.

The objection may properly be raised against the method proposed by Remy for the study, among other things, of the denitrifying power of soils, in that it makes use of qualitative methods in determining the disappearance of the nitrate in the culture solution, whereby the process is designated as denitrification. The chances of error here have been noted by Löhnis[d] and will become clear enough in view of the facts just stated. It should also be added that the disappearance of soluble nitrogen compounds from the soil (and culture solutions) as affected by bacterial activities is far-reaching enough to deserve careful study. A rapid multiplication of soil bacteria in the processes of ammonification, nitrification, and denitrification unavoidably involves the transformation of comparatively simple soluble nitrogen compounds into the insoluble nitrogen compounds of the bacterial bodies. The many millions of these under conditions favorable for their development may withdraw from circulation very large quantities of soluble nitrogen which does not become available again until the bacterial bodies are decomposed. In this manner a marked influence may be exerted on crop production.

In speaking of denitrification in its narrower sense, Stoklasa and Vitek[e] state that denitrification, where it occurs in the soil, plays at best only a subordinate part as compared with nitrification and ammonification. In their experiments it was found that in sugar-beet soils, benefited by applications of nitrate, the ammonification bacteria (the authors call them "ammonization bacteria") which, as is well known, convert the nitrate nitrogen into ammonia, prevail. They call attention especially to the great prevalence of *Clostridium*

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[a] Centbl. Bakt. [etc.], 2. Abt., 7 (1901), p. 609.

[b] Ibid., 14 (1905), p. 598.

[c] New Jersey Stas. Rpt. 1902, p. 183.

[d] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 8.

[e] Ibid., p. 187.

gelatinosum in the Bohemian sugar-beet soils, an organism which Laxa and Velich have found to be a common inhabitant on the roots of sugar beets. Their investigations fully confirm the results of these experimenters, and they found that *C. gelatinosum*, together with a whole series of other ammonification bacteria, like *Bacillus mycoides*, *B. subtilis*, *B. mesentericus vulgatus*, etc., plays an important part in the transformation of nitrate into ammonia nitrogen. They point out that with arabinose as a source of energy, *C. gelatinosum* changed 46 per cent of the nitrate nitrogen into ammonia, and used up almost 6 per cent of the total nitrogen for the synthesis of protein substances.

Thus we see from the studies of Stoklasa and Vitek that under certain conditions there may be an extensive but only partial reduction of nitrates in well-cultivated soils, reductions which do not necessarily diminish the total quantity of combined nitrogen in the soil. Stoklasa points out in another place[a] that sufficient allowance is not made for the action of nitrate applications on the bacteria in the soil. The addition of sodium nitrate strongly encourages the rapid development of certain bacterial species, which in turn affect the absorption and assimilation of the nitrate nitrogen by the crop.

For the sake of convenience Stoklasa arranges the various kinds of soil bacteria that affect the fortunes of soil nitrogen into seven groups, as follows: Group 1, bacteria which fix atmospheric nitrogen and cause it to form complex compounds; group 2, bacteria which decompose nitrogenous organic substances and produce ammonia as the end product; group 3, nitrous bacteria which oxidize ammonia to nitrous acid; group 4, nitrate bacteria which oxidize nitrites to nitrates; group 5, bacteria which reduce nitrates to nitrites and ammonia; group 6, denitrifying bacteria, which reduce a large portion of the nitrates to nitrites and the latter to elementary nitrogen; group 7, bacteria which may change ammonia, nitrites, and nitrates into protein bodies, or, generally speaking, into organic nitrogenous substances in the presence of organic acids or of carbohydrates. This last group includes members of all the other groups. In considering, therefore, the reactions in the soil, directly or indirectly produced by micro-organic life, we must take cognizance, not only of the main chemical transformations occasioned there, but also of the secondary reactions of which our knowledge is still quite limited. Stoklasa, Jelinek, and Ernest state[b] that they could find no appreciable loss of elementary nitrogen, due to denitrification, in Bohemia sugar-beet soils, although they observed the partial reduction of nitrates.

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[a] Bl. Zuckerrübenbau, 11 (1904), p. 321; abs. in Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 48.

[b] Ztschr. Zuckerindus. Böhmen, 30 (1906), p. 223.

In any case we should always consider the bacteriological processes in the soil, not as something apart, but only as an expression of general soil and climatic conditions. What these may be is well described by Wollny,[a] substantially as follows:

The main constituents of the soil differ considerably from one another in their relation to air, moisture, and temperature. During the portion of the season when decomposition proceeds most rapidly, quartz sand is the warmest of all soil constituents, and most accessible to atmospheric air, but it is also the driest. Clay is marked by its high moisture content, its insufficient ventilation, and its backwardness in warming up. Because of its great water-holding power, humus is not readily permeated by air, and warms up but slowly, hence it has a higher temperature only in the summer and early fall. Lime occupies an intermediate position between quartz sand and clay so far as its physical properties are concerned.

These properties of the main constituents of the soil indicate with sufficient clearness that the various soil factors which largely affect the decomposition of organic substances may either support or counteract one another, and the predominance of any one of them is determined by the quantitative relations. We thus come to the following considerations: The greater permeability and the more rapid warming up of the quartz sand are undoubtedly favorable for the decomposition of organic matter; on the other hand, its low water content is a retarding factor. Hence in soils of this character the supply of moisture is the controlling factor in the decomposition of organic substance, and since the proportion of moisture is here more directly dependent on timely precipitation the latter necessarily exerts an important influence on the decomposition processes. In a humid region, especially where the soil receives frequent additions of moisture, the breaking down of organic matter may proceed with great intensity, so rapidly in fact that under these conditions there may be no appreciable accumulation of humus in sandy soils. The same soils may behave quite differently in a dry climate, where there is a minimum supply of moisture, and the better aeration and higher temperature can further the destruction of the organic matter only so far as the slight amounts of moisture will permit. In this case decomposition proceeds more slowly but, excepting extreme conditions, still more rapidly than in other soil varieties.

There is usually no lack of water in clay soils for the decomposition of organic matter, but rather an insufficient quantity of air. Moreover, these soils are essentially cold soils. In consequence of this, the decomposition of organic matter in clay soils is determined, in the first place, by its permeability to air and, in the second place, by its temperature conditions. Under ordinary conditions, therefore, the decomposition of organic matter in clay soils proceeds quite slowly. These soils may, for this reason, be designated as "inactive," as compared with the "active" sandy soils. In humid regions very compact clay soils may exclude the air to such an extent that putrefaction, rather than decay, becomes prominent.

Hence most sandy soils are noted, as a rule, for their marked oxidation processes affecting not only the carbon, but also the ammonia derived from the organic material. Nitrification is quite energetic in such soils. In clay soils, on the other hand, the oxidation of organic matter is not only retarded, but is at times, and especially during periods of heavy rainfall, entirely discontinued. Under such conditions deoxidation processes become prominent, among them denitrification. Limestone soils may resemble one or the other of the foregoing, according to its mechanical constitution.

Humus soils exhibit considerable variations, determined by their water content. Where the humus is accumulated in great masses and is saturated with water, as for instance in swamps or bogs, putrefaction processes may prevail, thanks to the exclu-

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[a] Die Zersetzung der organischen Stoffe. Heidelberg: C. Winter, 1897, p. 158.

sion of air. When such soils are drained and exposed to the action of air, oxidation processes replace the deoxidation processes, and the decomposition of the organic matter, added to the soil, proceeds rapidly, especially under favorable seasonal conditions. The organic matter of the soil itself, because of the manner of its formation, does not decay as fast.

Arable soils made up of these constituents differ in their physical properties, according to the predominance of one or the other of these component materials, and hence exhibit corresponding differences in the decomposition of their organic matter.

The chemical constituents of the soil, as Wollny indicates, also affect the qualitative and quantitative character of its bacterial flora. Humus, clay, and lime carbonate may act chemically as well as physically, and variations in the amount of phosphates, sulphates, or chlorids, or in the amount of aluminum, iron, manganese, and magnesium compounds, may exert an appreciable influence. Furthermore, the character of the organic matter itself is of paramount importance, not merely as regards its concentration of nitrogen, but also as regards its content and relative proportions of the various nonnitrogenous organic substances.

BACTERIAL SYNTHESIS OF NITROGENOUS COMPOUNDS.

The breaking down of organic matter by bacteria involves, perforce, the growth and multiplication of the agents of decomposition. The bacterial cells, predominatingly nitrogenous in their composition, are built out of the more simple materials which are the by-products of decomposition. Hence in the decay of organic matter, which is essentially a process of simplification, there is formed a greater or smaller amount of highly organized bacterial substance. In other words, synthesis and analysis proceed hand in hand. For instance, in the ammonification of peptone or albumin there is always formed some new protein substance, in the construction of which a portion of the energy set free in the breaking down of the organic food is utilized. Similar formation of complex substances occurs in the case of nitrification, denitrification, sulphate reduction, etc. In some instances the amount of bacterial protein thus formed, as compared with the total amount of nitrogen present, may be quite small, as in the case of the destruction of nitrates by *Bacillus* (*Pseudomonas*) *pyocyaneus*. In other instances a large proportion of the simple nitrogenous substances may be converted into protein bodies, as in the case of certain soil organisms, which may convert nitrate nitrogen into protein nitrogen almost quantitatively in the presence of carbohydrates. The same is true also of molds, which in acid soils rapidly convert the nitrogen of ammonium salts into insoluble protein nitrogen. We see thus that in all decomposition processes, where the analytical reactions prevail, the synthetical processes are never wholly wanting, and under certain conditions these may play an important part in the nitrogen feeding of crops.

NITROGEN FIXATION.

The various bacteriological activities already considered deal with the transformation of soil nitrogen. Starting with the complex protein molecules in the plant and animal residues that are to undergo decomposition and are again to be rendered available as plant food, there are many bacterial species concerned in these processes of transformation. But whatever the complexity of such processes, whatever the interrelation or the interdependence of the several bacterial factors, they can add nothing to the store of soil nitrogen. Whatever the rate of peptonization, of ammonification, of nitrification, or of denitrification, the soil nitrogen can not thereby increase in amount, but it may suffer a serious decrease, as has been stated. It has been shown [a] how enormous such losses may become, and how rapidly even the richest soils may be depleted of their nitrogen store. Fortunately, there are compensating factors. Just as the nitrogen atoms may be torn apart from those of other elements and sent back into the atmosphere as free nitrogen gas, so may the latter be made to combine with other elements in the formation of new nitrogen compounds. These compensating factors, that deal not with the mere transformation of soil nitrogen but with the addition to its store, are the nitrogen-fixing bacteria.

The fact that uncultivated soils when left to themselves can increase their store of fertility has been known for generations. Indeed, the custom of growing one crop in three, five, seven, or ten years, as the case may be, and abandoning the land to weeds during the interval, was known in Europe and Asia centuries ago. Far less ancient is the knowledge that such fertility accumulation is practically all due to the nitrogen increase in the abandoned soils, and still more recent is the knowledge that such nitrogen gains in the soil are largely due to the work of micro-organisms.

Boussingault wrote, in 1858, as follows:

> Vegetable earth contains not only dead organic matter, but living organisms—germs, the vitality of which is suspended by drying and reestablished under favorable conditions as to moisture and temperature. This mycodermic vegetation is not always visible to the naked eye, and its progress must be followed by the aid of the microscope. The mycoderms have only an ephemeral existence, and they leave their detritus in the soil, which in time may give rise to ammonia and to nitric acid. Even if the nitrogen of the air takes part in nitrification, a part of the nitrogen will exist in mycoderms and their remains.[b]

Boussingault had in his hands facts which might have revealed to him the cause of the nitrogen gains in his soils had contemporary information been sufficient to throw greater light on the subject. Hellriegel and Wilfarth's discovery of symbiotic fixation was still almost

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[a] Snyder, Minnesota Sta. Bul. 89, p. 193.

[b] As cited from Gilbert by Lipman, U. S. Dept. Agr., Bur. Chem. Bul. 81, p. 146.

thirty years in the future, Berthelot's study of nitrogen-fixing bacteria, Winogradski's isolation of nitrifying bacteria and of the nitrogen-fixing *Clostridium pasteurianum* were not to come for more than three decades, and, in fact, the technique of modern bacteriology was practically unknown.[a] In the time that has since elapsed we have learned to know of several groups of soil organisms capable of fixing gaseous atmospheric nitrogen, and we have learned to distinguish between nitrogen fixation by the bacteria living within the soil itself and those living within the root tubercles of higher plants. For the sake of convenience, the former will be designated as nonsymbiotic and the latter as symbiotic nitrogen-fixing bacteria.

NONSYMBIOTIC FIXATION.

Soon after the establishment of the experiment station at Meudon, France, in 1883, Berthelot[b] undertook the study of soils, particularly in their relation to free and combined nitrogen. As a result of these studies he was the first to recognize intelligently that gains do take place in bare unsterilized soils, and that the gain is due to microscopic organisms in the soil. Without going into further detail, it is sufficient to state here that corroborative evidence as to the fixation of nitrogen by soils and soil organisms was also furnished by Julie,[c] Dietzell,[d] Tacke,[e] Gautier and Drouin,[f] Schloesing and Laurent,[g] Frank,[h] Koch and Kossovich,[i] Krüger and Schneidewind,[j] Richter,[k] Petermann,[l] Immendorff,[m] Dehérain,[n] Pagnoul,[o] Pichard,[p] Bréal,[q] and Kühn.[r]

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[a] Lipman, Ibid., p. 147.

[b] Chimie Vegetale et Agricole. Paris: Masson et Cie., 1899.

[c] Bul. Soc. Agr. France, 1886, No. 1, pp. 19–29; Compt. Rend. Acad. Sci. [Paris], 101 (1885), p. 1008; Ann. Agron., 12 (1886), p. 5.

[d] Tagebl. 57. Versamml. Deut. Naturf. u. Aerzte, Magdeburg, 1884, p. 176.

[e] Landw. Jahrb., 18 (1889), p. 439.

[f] Compt. Rend. Acad. Sci. [Paris], 106 (1888), pp. 754, 863, 944, 1098, 1174, 1232, 1605; 113 (1891), p. 820.

[g] Ibid., 106 (1888), pp. 805, 898, 982, 1123; 107 (1888), p. 290; 109 (1889), pp. 210, 345; 111 (1890), p. 750; 113 (1891), pp. 776, 1059; 115 (1892), pp. 659, 732.

[h] Landw. Jahrb., 17 (1888), p. 421; 19 (1890), p. 588.

[i] Bot. Ztg., 51 (1893), 2. Abt., p. 321; 52 (1894), p. 97.

[j] Landw. Jahrb., 28 (1899), p. 579; 29 (1900), p. 771; 30 (1901), p. 633.

[k] Landw. Vers. Stat., 51 (1899), p. 221.

[l] Bul. Acad. Roy. Sci. Let. et Beaux Arts Belg., 3. ser., 25 (1893), p. 267; abs. in Chem. Centbl., 1893, I, p. 988.

[m] Landw. Jahrb., 21 (1892), p. 281.

[n] Compt. Rend. Acad. Sci. [Paris], 125 (1897), p. 278.

[o] Ann. Agron., 16 (1890), p. 250.

[p] Compt. Rend. Acad. Sci. [Paris], 114 (1892), p. 81.

[q] Ann. Agron., 18 (1892), p. 369.

[r] Fühling's Landw. Ztg., 50 (1901), p. 2; abs. in Centbl. Bakt. [etc.], 2. Abt., 7 (1901), p. 601.

Not content with proving that there may be an accumulation of nitrogen in bare and uncropped soils, Berthelot also attempted[a] to isolate the specific organisms to which such fixation is due. With the aid of Guignard he made inoculations in sterile bouillon from a garden soil, and after incubation at 20° C. and proper dilution with sterile bouillon, gelatin plates were prepared. Inoculations were made from the colonies which appeared on the plates and the bacteria thus isolated were tested for their nitrogen-fixing power. A careful description of these organisms is lacking, so that their identification is impossible. Berthelot gives, however, some general statements concerning them, and, what is more important, shows by his analytical results that there was a gain of combined nitrogen in some cases. He concludes from these experiments that there are chlorophylless bacteria in the soil capable of fixing elementary atmospheric nitrogen. They require organic carbon and hydrogen and enough combined nitrogen to promote their initial growth. When the amount of combined nitrogen becomes considerable, the bacteria prefer to draw their nitrogen supply from the combined soil nitrogen, and for this reason the fixation of atmospheric nitrogen has its limits. It is quite evident from this that Berthelot had cleared the ground for more exact bacteriological studies. To him belongs the credit of having proved conclusively that the fixation of gaseous atmospheric nitrogen may be accomplished in arable soils by virtue of certain micro-organisms contained in them. His skill as a chemist enabled him to overcome the many difficulties involved in the analytical work and to render his researches of permanent interest.[b]

At this point the problem was systematically attacked by Winogradski.[c] His first communication dates back to 1893, and deals with experiments that were carried out with crude cultures of a butyric ferment. The nutritive solutions employed by him were carefully freed from the last traces of nitrogen, and contained, besides the mineral salts, a fermentable organic compound, dextrose. Butyric fermentation was produced in these solutions by a long spore-bearing bacillus which developed normally in the absence of combined nitrogen. Winogradski had not succeeded at this time in isolating this bacillus in pure culture. In his earlier experiments it was accompanied by two other bacilli. It seemed, however, that the latter took no part in the fixation of free nitrogen, since they refused to grow in media containing no combined nitrogen, but grew vigorously in solutions to which small quantities of ammonium salts were added. This, taken together with the fact that they produced neither gas nor butyric acid, led Winogradski to believe that these associated bacteria were incapable of fixing free

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[a] Compt. Rend. Acad. Sci. [Paris], 116 (1893), p. 842.

[b] Lipman, J. G., U. S. Dept. Agr., Bur. Chem. Bul. 81, p. 149.

[c] Compt. Rend. Acad. Sci. [Paris], 116 (1893), p. 1385.

nitrogen, although capable of growing in solutions very poor in combined nitrogen. Quantitative determinations showed that the maximum fixation was attained where no combined nitrogen was purposely added; namely, with 3.1 milligrams of nitrogen for every gram of dextrose used. When combined nitrogen was added to the culture solution the fixation of nitrogen was diminished. Thus with 2 grams of dextrose and 1.8 milligrams of combined nitrogen in the solution there was 1.7 milligrams of nitrogen fixed, while with 4 grams of combined nitrogen added the fixation was only 0.6 milligram of nitrogen.

Subsequently, Winogradski[a] worked with pure cultures of the butyric ferment. He isolated the latter by anaerobic culture methods, and found that aerobic growth can take place only in the presence of certain aerobic bacteria which serve to diminish the oxygen pressure. With the two associated bacteria, and the butyric ferment, he noted a diminution in the amount of nitrogen fixed as the concentration of the dextrose solution or the amount of initial nitrogen increased. When the amount of combined nitrogen added reached 21.2 milligrams for every gram of dextrose there was even a loss of nitrogen (2.2 milligrams). Winogradski was, therefore, led to conclude that the gain of nitrogen depends on the ratio of the combined nitrogen to the sugar. In order that any gain may be obtained the ratio of combined nitrogen to the dextrose should be 6:1,000. Because of the characteristic formation of clostridia, Winogradski named this bacillus *Clostridium pasteurianum*.[b]

We thus pass from a vague belief in the possibility of nitrogen fixation by soil bacteria to the certainty that such organisms exist, and thence to even the more definite knowledge of carefully described specific nitrogen-fixing bacteria. To this knowledge there was soon added a very interesting chapter in the history of nitrogen fixation, originating in the investigations of Caron,[c] owner of the Ellenbach estate in Germany. Caron observed in plating various soil samples that the soils from under leafy crops contained greater numbers of bacteria than soils from under gramineæ. He found that the bacterial flora in the spring was different from that in the fall, and noted the greatest increase in numbers in soil samples from a summer fallow. Cultures were made in bouillon and on gelatin of the organisms which were encountered most frequently and of those which grew best at low temperatures. Pure cultures were used for vegetation experiments, carried out with pots to which 40 cubic centimeters of bouillon culture or of sterile bouillon was applied.

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[a] Compt. Rend. Acad. Sci. [Paris], 118 (1894), p. 353.

[b] Arch. Sci. Biol. [St. Petersb.], 3 (1895), No. 4, p. 297; Centbl. Bakt. [etc.], 2. Abt., 9 (1902), p. 43; 10 (1903), p. 514.

[c] Landw. Vers. Stat., 45 (1894), p. 401.

The yields thus obtained were usually in favor of the inoculated pots, but showed considerable variations from season to season. In one case, for instance, the yield of the inoculated to the uninoculated was in the ratio of 139:100, and in another case, with mustard as the crop, it was 195:100. Particularly favorable results were secured with a spore-bearing bacillus, which he named later *B. ellenbachensis*.

Caron's investigations soon attained considerable notoriety, and led to the commercial exploitation of his cultures. A commercial firm of Elberfeld, Germany, placed on the market a bacterial preparation under the name of "Alinit." The preparation, the manufacturers claimed, would enable nonleguminous crops to make use of free atmospheric nitrogen, provided certain precautions were observed. Naturally enough, the matter created much discussion in Germany and elsewhere. Alinit was hailed with enthusiasm as an acceptable substitute for the expensive nitrogenous fertilizers, and to most chemists and bacteriologists it seemed well worthy of investigation. It was widely recognized that the matter was of great moment to agriculture, for it held in it the promise of a simple solution of a vexed problem.

Among the first to study the properties of Alinit was Stoklasa.[a] His studies led him to conclude that Alinit was a dried and ground culture of *Bacillus megatherium* De Bary, eminently capable not only of rendering soluble the inert soil nitrogen, but also of fixing considerable quantities of gaseous nitrogen.[b] Many other investigators, among them Lauck,[c] Hartleb,[d] Stutzer and Hartleb,[e] Kolkwitz,[f] Gain,[g] Lutoslawski,[h] Schulze,[i] Krüger and Schneidewind,[j] and Severin,[k] studied the Alinit bacillus with results usually negative, and with no evidence to support Stoklasa's extravagant claims. It has also been demonstrated that the Alinit bacillus is not identical with *B. megatherium*, although it belongs to the same group of organisms. In fact, Severin found in commercial samples of Alinit two organisms very closely resembling one another, and designated them as *B. ellenbachensis* α and β.

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[a] Centbl. Bakt. [etc.], 2. Abt., 4 (1898), pp. 39, 78, 119, 284, 507, 535.

[b] Ibid., 5 (1899), p. 350; 6 (1900), p. 22.

[c] Ibid., 4 (1898), p. 290.

[d] Ibid., 5 (1899), p. 706.

[e] Ibid., 4 (1898), pp. 31, 73.

[f] Ibid., 5 (1899), p. 670.

[g] Rev. Gén. Bot., 11 (1899), pp. 18–28; abs. in Centbl. Bakt. [etc.], 2. Abt., 5 (1899), p. 847.

[h] Deut. Landw. Presse, 25 (1898), No. 87, p. 920.

[i] Landw. Jahrb., 30 (1901), p. 319.

[j] Ibid., 28 (1899), p. 579.

[k] Centbl. Bakt. [etc.], 2. Abt., 9 (1902), pp. 712, 746.

Briefly stated, the results obtained with Alinit, both in the field and laboratory, give but little confirmation of the claims which its friends advanced. It has been the cause of much study, and of more discussion, and when the evidence is summed up it must be admitted that Alinit does not make accessible to higher plants the free nitrogen of the air.

An important addition to the history of nonsymbiotic nitrogen fixation was made by Beijerinck in 1901.[a] His discovery included a new group of large aerobic bacilli which he named Azotobacter, two species of which he described, viz, *Azotobacter chroococcum* and *A. agilis.* A third species, *A. vinelandii,* was added to the list by one of the writers in 1903,[b] and two more, *A. beijerincki,* and *A. woodstownii,* in the following year.[c] In a subsequent paper published jointly with Van Delden,[d] Beijerinck asserted that *A. chroococcum* is incapable of fixing any appreciable quantities of nitrogen when growing in pure culture. Large amounts of nitrogen are fixed only when it is growing together with other organisms, either spore bearing or nonspore bearing. The former are members of the Granulobacter group; the latter include some members of the aerogenes group, and also an organism not hitherto described which the authors named *B. radiobacter.* They attributed to Granulobacter the power to fix atmospheric nitrogen, even when alone, but this power becomes very pronounced only when it is growing together with *A. chroococcum* On the other hand, aerogenes and radiobacter have not themselves the power to fix free nitrogen. Hence when growing together with *A. chroococcum* they either acquire that power, or *A. chroococcum* itself acquires the power, or, it may be, both acquire the power. Gerlach and Vogel, on the other hand,[e] proved conclusively that *A. chroococcum* is capable of fixing large quantities of atmospheric nitrogen even when growing in pure culture. They disagree with Beijerinck and Van Delden's statement and are of the opinion that the Azotobacter group, and especially *A. chroococcum* isolated by Beijerinck and by themselves, is capable of fixing by itself considerable quantities of nitrogen.[f] They add elsewhere[g] that a series of other experiments to account for the results obtained by Beijerinck and Van Delden had failed thus far to give satisfactory

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[a] Verslag. K. Akad. Wetensch. Amsterdam, 9 (1900–1901), p. 633; 10 (1901–2), p. 8; Centbl. Bakt. [etc.], 2. Abt., 7 (1901), p. 561.

[b] Lipman, J. G. Nitrogen-Fixing Bacteria. Doctor's Thesis, Cornell University, June, 1903.

[c] New Jersey Stas. Rpt. 1904, p. 237.

[d] Centbl. Bakt. [etc.], 2. Abt., 9 (1902,) p. 3.

[e] Ibid., 8 (1902), p. 669; 9 (1902), pp. 817, 881.

[f] Gerlach and Vogel. Centbl. Bakt. [etc.], 2. Abt., 9 (1902), p. 820.

[g] Ibid., p. 882.

results. Freudenreich, who also studied *A. chroococcum* says that his own experiments tend to support the view held by Gerlach and Vogel,[a] Heinze,[b] Koch,[c] and one of the writers[d] also found that members of the Azotobacter group can fix atmospheric nitrogen when growing in pure culture, thus confirming the observation of Gerlach and Vogel, and rendering unnecessary Beijerinck and Van Delden's elaborate explanation. Moreover, it was demonstrated in the laboratories of the New Jersey Experiment Station why the latter were led to conclude that *A. chroococcum* is by itself incapable of fixing atmospheric nitrogen.[e]

It should be noted here that the term nonsymbiotic as applied to the nitrogen-fixing organisms considered above is not, strictly speaking, correct. Reinke[f] showed, for instance, that Azotobacter organisms may live in symbiosis with marine algæ, and with volvox, and Fischer[g] observed somewhat similar relations between Azotobacter and Oscillaria. These observations throw a new light on the earlier studies of Gautier and Drouin, Schloesing and Laurent, Frank, Koch and Kossowich, Krüger and Schneidewind, and Richter (see p. 77), all of whom noted gains of nitrogen in soils covered with algæ, and amply confirm Kossowich's[h] conclusion that algæ can cause an increase in the store of soil nitrogen only when growing together with bacteria.

It seems certain also that nitrogen fixation by members of the Azotobacter group is enhanced by the presence of other bacteria, as was well demonstrated by Beijerinck,[i] and also by one of the writers.[j] In view of these facts it must be granted that there may be symbiotic fixation of nitrogen by microscopic organisms living within the soil itself, and their designation as nonsymbiotic has only a relative value, serving as a distinction from the symbiosis between *Pseudomonas radicicola* and higher plants.

There are, besides the two great groups represented by *Clostridium pasteurianum* and *Azotobacter chroococcum*, respectively, other soil organisms which possess a variable, but frequently very distinct, nitrogen-fixing power. One need but recall Beijerinck's observation concerning a freshly isolated culture of *Bacillus mesentericus*,[k]

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[a] Gerlach and Vogel. Centbl. Bakt. [etc.], 2. Abt., 10 (1903), p. 519.

[b] Ibid., p. 674.

[c] Ibid., 13 (1904–5), p. 111.

[d] New Jersey Stas. Rpt. 1903, p. 247.

[e] Ibid., 1904, p. 262.

[f] Ber. Deut. Bot. Gesell., 21 (1903), pp. 371, 481.

[g] Centbl. Bakt. [etc.], 2. Abt., 12 (1904), p. 267.

[h] Bot. Ztg., 52 (1894), 1. Abt., p. 97.

[i] Centbl. Bakt. [etc.], 2. Abt., 9 (1902), p. 3.

[j] New Jersey Stas. Rpt. 1903, p. 258.

[k] Centbl. Bakt. [etc.], 2. Abt., 9 (1902), p. 33.

which proved capable of fixing very appreciable quantities of nitrogen. Similarly the more recent studies of Löhnis,[a] demonstrating the ability in this direction of *Bacterium pneumoniæ, B. lactis viscosum, B. radiobacter,* and *B. prodigiosum,* bear on this point, as do the studies by one of the writers on the nitrogen-fixing power of *Bacillus pyocyaneus.*[b] Furthermore, it is well established by this time that *P. radicicola* can fix appreciable quantities of nitrogen when growing outside of the legume tubercles. Ample evidence in this direction is furnished by the investigations of Mazé,[c] Chester,[d] Moore,[e] and Löhnis.[f] We see thus that the power of fixing atmospheric nitrogen is not limited to a few species, as Winogradski had at one time supposed, and it is safe to predict, that with our growing knowledge of soil bacteriological processes, other species will be added to the already large list.

As to the interrelation of the various nitrogen-fixing organisms in the soil our information is extremely limited. It is known, for instance, that Azotobacter species are widely distributed,[g] and the same is true of the butyric ferments. In mannite cultures from arable soils, the two are usually found together, but frequently only butyric fermentation occurs in such solutions, a fact familiar to all students of the subject. Löhnis[h] noted that Azotobacter membranes are more readily obtained in winter than in summer, and Fischer[i] showed that limed soils may readily yield a good Azotobacter growth where the same soils unlimed fail to produce any Azotobacter growth whatsoever. There is no doubt at any rate that under proper conditions the Azotobacter and other species may materially increase the nitrogen content of the soil. An indication, at least, as to the extent of such gains is furnished by Kühn,[j] who grew nonleguminous crops on certain soils for twenty years and noted no diminution in the yield of rye, even though only nonnitrogenous mineral fertilizers were applied. No less instructive is the observation of Hall[k] on the nitrogen gains in Broadbalk and Geescroft fields at Rothamsted abandoned to themselves between 1882 and 1904, the former showing an annual gain of more than 100 pounds

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[a] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 582.

[b] New Jersey Stas. Rpt. 1902, p. 229.

[c] Ann. Inst. Pasteur, 11 (1897), p. 44.

[d] Delaware Sta. Bul. 66.

[e] U. S. Dept. Agr., Bur. Plant Indus. Bul. 71, p. 31.

[f] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 594.

[g] Heinze. Centbl. Bakt. [etc.], 2. Abt., 12 (1904), p. 57, footnote.

[h] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 583.

[i] Ibid., p. 33.

[j] Fühling's Landw. Ztg., 50 (1901), p. 2; abs. in Centbl. Bakt. [etc.], 2. Abt., 6 (1901), p. 601.

[k] Jour. Agr. Sci., 1 (1905), No. 2, p. 241.

of nitrogen per acre, the latter an annual gain of rather more than 25 pounds per acre. Concerning this phenomenon, Hall asks:

> How comes it that the Geescroft land, with no plants growing on it which are capable of fixing free nitrogen, has yet gained an enormous quantity of nitrogen during the twenty years under review, a quantity which at the lowest reckoning amounts to about 25 pounds per acre per year? The nitrogen brought down in the rain would account for perhaps 5 pounds per acre per annum, a little more will come in the form of dust, bird droppings, and other casual increments, while some may be due to fixation of atmospheric nitrogen by bacteria in the soil not associated with leguminous plants, like the *Azotobacter chroococcum* of Beijerinck and Winogradski's *Clostridium pasteurianum*. The Azotobacter has been found abundantly in the Rothamsted soils, and as in the case of grass land like the present the decaying vegetation would supply the carbohydrate which the bacterium must oxidize in order to fix nitrogen, it is quite possible that it may have effected considerable gains of nitrogen.

Hall apparently overlooks the fact that the actual gains of nitrogen were even greater than those indicated by the analyses, for considerable amounts must have been lost in the course of decay and nitrification, and the final gains, therefore, as noted by Hall, represent only the algebraic sum of the total gain and loss.

Our own experiments[a] furnish still further proof of the possible extent of nonsymbiotic nitrogen fixation, the gains in these experiments amounting in some cases to more than one-third of the nitrogen originally present or supplied, and that in the course of only two short growing seasons. It is likewise probable that the beneficial effect of fallowing and the nitrogen gains observed are largely due to Azotobacter species, Hiltner and Störmer having already noted in their soil bacteriological studies that the decrease in the number of organisms capable of growing on gelatin can scarcely be accounted for otherwise than by the assumption that they are suppressed by a special species or group of organisms that do not grow on gelatin, but to whose activities the favorable effects of fallowing are largely due.[b]

More recent experiments with pure or mixed cultures of Azotobacter and of *Clostridium pasteurianum* by Thiele,[c] Warmbold,[d] Haselhoff and Bredemann,[e] and Schneider,[f] confirm the earlier experiments as to the ability of these organisms to increase the store of combined nitrogen in the soil.

The earlier observations as to the wide distribution of Azotobacter are amplified by the experience of Perotti,[g] who found them in soil

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[a] New Jersey Stas. Bul. 180.

[b] Studien über die Bakterienflora des Ackerbodens, etc. Berlin, 1903, p. 544. Reprinted from Arb. K. Gsndhtsamt., Biol. Abt., 3 (1903).

[c] Landw. Vers. Stat., 63 (1905–6), p. 161.

[d] Landw. Jahrb., 35 (1906), p. 1.

[e] Ibid., p. 381.

[f] Ibid., Sup. 4, p. 63.

[g] Atti. R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., 5. ser., 15 (1906), I, p. 295.

samples from different parts of Italy. Stoklasa and his associates,[a] who also studied cultures of Azotobacter, ascribe the nitrogen-fixing power of these organisms to their ability to produce glycolytic enzyms. By means of these enzyms the glucose or mannite in the culture solution is broken down with the formation of such intermediary products as alcohol, lactic acid, acetic acid, and butyric acid. The gaseous products include large quantities of carbon dioxid and slight amounts of hydrogen. The authors believe that the elementary hydrogen formed in the decomposition of the carbohydrates by Azotobacter plays an important rôle in the chemistry of nitrogen fixation.

Christensen[b] made extensive study of Azotobacter in its relation to changing soil conditions. The facts gathered by him again emphasize the important rôle of calcium carbonate and of other basic substances in the life processes of the Azotobacter species.[c] This dependence of the organisms on lime and magnesium carbonates in the soil is so characteristic as to suggest its utilization as a means for the measure of the lime requirements of soils. The Azotobacter species could probably be utilized in a similar manner for the study, also, of the phosphorous requirements of soils, as has been suggested elsewhere by one of the writers.[d] The experiments of Christensen brought out, among other results, the very interesting fact that Azotobacter cultures could derive their calcium from dibasic calcium phosphate and from calcium salts of organic acids, as well as from calcium carbonate. They could not, on the other hand, utilize the calcium of tribasic calcium phosphate, or of the chlorid or sulphate. Potassium and sodium phosphates, dibasic calcium phosphate, and Thomas slag offered readily accessible sources of phosphorus; whereas the phosphates of iron and aluminum, pure tribasic calcium phosphate and bone ash served only as difficultly available sources of phosphorus. Raw mineral phosphates and bone meal failed entirely to furnish enough available phosphorus for the development of the Azotobacter. Christensen concludes[e] that this variable relation of the Azotobacter growth toward the different lime salts and phosphates seems to justify the hope that it will become possible by the determination of the bacterial food requirements, to secure a general expres-

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[a] Ber. Deut. Bot. Gesell., 24 (1906), p. 22; also Ztschr. Ver. Deut. Zuckerind, 1906, p. 815.

[b] Centbl. Bakt. [etc.], 2. Abt., 17 (1906), pp. 109, 161, 378.

[c] See Fischer, Hugo. Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 33; 15 (1906), p. 235.
Heinze. Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 174, footnote.
Lipman. New Jersey Stas. Rpt. 1904, p. 262.

[d] Lipman. New Jersey Stas. Rpt. 1906.

[e] Centbl. Bakt. [etc.], 2. Abt., 17 (1906), p. 383.

sion for the content in the soil of the plant food available to crops. The observation of Christensen just cited is in agreement with the similar statement made independently by one of the writers in connection with the experiments reported some time ago.[a]

Everything considered, we are still quite uncertain as to the true position of these bacteria so far as practical agriculture is concerned. This uncertainty is voiced by Thiele, who says:

> We have unequivocally established the ability of Azotobacter organisms to fix atmospheric nitrogen in the laboratory, but we do not know as yet whether this ability manifested by the organisms under artificial conditions is a specific characteristic, as is the production of alcohol by yeast. It is not impossible that the Azotobacter organisms are brought in the laboratory to a condition of starvation, which compels them to make use of their inherent ability to gather atmospheric nitrogen; or that by additions of large quantities of organic matter they are placed in so favorable a situation as to stimulate their latent (under ordinary conditions) ability of nitrogen fixation; there is at least an analogy here to the manifestation by yeast in peptone media of unusual characteristics. * * *
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> Their action in the soil is thus far quite unknown to us. We have advanced theories, but have not yet furnished the proofs, and shall be unable to furnish these until we have devised methods more accurate than those used at present for the detection of the slight variations in the nitrogen content of soils. * * * Thus the life and activity of Azotobacter remain for us an almost unsolved puzzle, and it would be well for the practical farmer not to anticipate the time when he can replace an application of sodium nitrate by inoculation with Azotobacter. Before that time arrives we shall have to illuminate the dark field before us by extensive research.

Thiele is quite right in his statements, so far as our limited knowledge of the activities of the Azotobacter species in actual field practice is concerned. He shares in this respect the views held by Pfeiffer.[b] Nevertheless the views of these two investigators are unnecessarily extreme. There are a number of experiments and observations on record, some of them already noted in the foregoing pages, that show unmistakably enough extensive gains of combined nitrogen where the influence of leguminous crops was excluded. As Vogel[c] has observed, Pfeiffer's statements can only be regarded as subjective and advanced from the standpoint of the agricultural chemist. They are not sufficient to discount the extensive experience of practical farmers and the observations of agricultural bacteriologists all pointing toward nitrogen fixation by free living soil bacteria. Vogel also cites the observations of Vibrans, Schneidewind, Caron, Löhnis, and others, which indicate that by proper methods of soil treatment, among them suitable methods of fallowing, heavy loam and clay soils may be made to increase their content of nitrogen. To sum it up, therefore, the knowledge already in our possession encourages us in the

[a] New Jersey Stas. Rpt. 1906, p. 177.

[b] Stickstoffsammelnde Bakterien, Brache und Raubbau. Berlin: Paul Parey, 1904.

[c] Centbl. Bakt. [etc.], 2. Abt., 15 (1905), p. 35.

belief that the aerobic and anaerobic nitrogen-fixing bacteria, because of their universal distribution and their pronounced ability to form nitrogen compounds out of elementary nitrogen, are not an unimportant factor in maintaining our supply of combined nitrogen, and that future research will teach us to make intelligent use of these organisms in successful crop production.[a]

SYMBIOTIC FIXATION.

The cumulative evidence of many centuries fixed in the consciousness of the farmer the firm belief that certain leguminous crops have the power somehow to enrich the soil. This belief found its practical application in olden times, and persisted at a period in the nineteenth century when it seemed contrary to scientific authority. The

[a] Other references on nonsymbiotic fixation, not cited in the text, include:
Henry. Ann. Sci. Agron., 8 (1903), p. 313.
Sestini. Landw. Vers. Stat., 60 (1904), p. 103.
Löhnis. Deut. Landw. Presse, 31 (1904), p. 817.
von Rümker. Der Boden und seine Bearbeitung. Berlin: Paul Parey, 1904, 2. ed. (Tagesfragen aus dem modernen Ackerbau, No. 1.)
Vogel. Fühling's Landw. Ztg., 52 (1903), pp. 178, 213.
Benecke and Keutner. Ber. Deut. Bot. Gesell., 21 (1903), p. 333.
Keutner. Wiss. Meeresunters., n. ser., 8 (1905), Abt. Kiel, p. 37.
Gerlach. Jahrb. Deut. Landw. Gesell., 17 (1902), p. 20.
Hiltner. Deut. Landw. Presse, 28 (1901), pp. 203, 212, 231.
Remy. Illus. Landw. Ztg., 23 (1903), pp. 983, 993, 1004, 1014.
Remy. Mentzel u. von Lengerke's Landw. Kalender, 56 (1903), pt. 2, p. 59.
Hiltner. Arb. Deut. Landw. Gesell., 1904, No. 98, p. 59.
Behrens. Arb. Deut. Landw. Gesell., 1901, No. 64, p. 108.
Buhlert. Fühling's Landw. Ztg., 52 (1903), pp. 451, 494.
Muth. Verhandl. Naturw. Ver. Karlsruhe, 16 (1902–3), p. 69.
Stutzer. Deut. Landw. Presse, 31 (1904), pp. 73, 81, 89, 137, 157.
Behrens. Mitt. Deut. Landw. Gesell., 1904, p. 181.
Jacobitz. München. Med. Wchnschr., 49 (1902), p. 1504.
Süchting. Amtsbl. Landw. Kammer Kassel, 1905, No. 4; reprinted in Hanover. Land. u. Forstw. Ztg., 58 (1905), p. 62; abs. in Deut. Landw. Presse, 32 (1905), No. 25, p. 225.
Jacobitz. Ztschr. Hyg., 45 (1903), p. 97.
Brefeld. Jahresber. Schles. Gesell. Vaterländ. Cult., 78 (1900), 2. Abt., Sitzber. Zool.-Bot. Sek., Nov. 15, 1900, p. 27.
Ternetz. Ber. Deut. Bot. Gesell., 22 (1904), p. 267; Centbl. Agr. Chem., 34 (1905), p. 205.
Bouilhac and Giustiniani. Compt. Rend. Acad. Sci. [Paris], 138 (1904), p. 293.
Gerlach. Illus. Landw. Ztg., 24 (1904), pp. 37, 59.
Vogel. Centbl. Bakt. [etc.], 2. Abt., 15 (1905), pp. 33, 174, 215.
Jacobitz. Centbl. Bakt. [etc.], 2. Abt., 7 (1901), pp. 783, 833, 876.
Koch, A. Mitt. Deut. Landw. Gesell., 21 (1906), p. 111; 22 (1907), p. 117.
Heinze, B. Centbl. Bakt. [etc.], 2. Abt., 16 (1906), pp. 640, 703.
Pringsheim, H. Centbl. Bakt. [etc.], 2. Abt., 16 (1906), p. 795.

earliest recorded observations[a] on the root tubercles of legumes did not connect the presence of these root swellings with the reputed soil-enriching qualitites of the legumes. To Malpighi himself they were only root galls, and to the earlier students of the nineteenth century they appeared either as pathological processes[b] caused by fungi, or merely as modifications of the normal roots.[c] Careful studies of the legume tubercles led Woronin[d] to declare in 1866 that he had found in them small organized bodies that were probably bacteria, and he regarded the tubercles as pathological outgrowths. Eriksson[e] also observed the vibrio-like bodies noted by Woronin and found, besides, fungus hyphæ which passed from cell to cell, dividing into finer branches as they grow toward the center of the tubercle. Eriksson observed, likewise, that the vibrio-like bodies were not always rod-shaped, but became modified at times into forked forms. Frank[f] found in 1879 that tubercles are seldom absent on the roots of legumes, whether native or foreign, an observation in harmony with those of Treviranus,[g] Lachmann,[h] and Wydler.[i] Frank also showed that the formation of tubercles could be prevented by sterilization of the soil. He succeeded in this manner in preventing tubercle formation on pea seedlings, and proved thus that the tubercles owe their origin to microbes living in the soil. The belief that tubercle formation is due to outside infection had been expressed in the previous year by Kny,[j] who failed to find tubercles on plants grown in water culture. The same view as to the origin of the tubercles was held by Prillieux.[k] Subsequently Frank[l] changed his earler views as to the origin of legume tubercles and accepted those of his pupil Brunchhorst,[m] who regarded the tubercles as food reservoirs, and their bacteria-like

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[a] Malpighi. Opera omnia, 1687, vol. 2, p. 126.

[b] De Candolle. Prodromus II, 1825; also Mémoires sur la famille des légumineuses. Paris: A. Belin, 1825.

[c] Kolaczek. Lehrbuch der Botanik. Wien: Braunmüller, 1856, p. 374.

Gasparini. Observazioni sulla struttura dei tuberculi spongiolari di alcune piante leguminosi. Atti Accad. Sci. [Napoli], 6 (1851), p. 221.

[d] Mem. Acad. Imp. Sci. St. Petersb., 7. ser., 10 (1866), No. 6; Ann. Sci. Nat., Bot., 5. ser., 7 (1867), p. 73.

[e] Studier öfver Leguminosernas Rotknölar. Inaug. Diss., Lund, 1874. Bot. Ztg., 32 (1874), p. 381.

[f] Bot. Ztg., 37 (1879), pp. 376, 393.

[g] Bot. Ztg., 11 (1853), p. 393.

[h] Landw. Mitt., Ztschr. K. Landw. Lehranst. Poppelsdorf, 1858, No. 1; reprinted in Centbl. Agr. Chem., 20 (1891), p. 837.

[i] Flora, n. ser., 18 (1860), p. 17.

[j] Zitzber. Bot. Ver. Prov. Brandenburg, 1878, Apr. 26, p. 55.

[k] Bul. Soc. Bot. France, 26 (1879), p. 98.

[l] Deut. Landw. Presse, 13 (1886), p. 629.

[m] Ber. Deut. Bot. Gesell., 3 (1885), p. 241.

bodies, which he named "bacteroids," as products of the plant cells formed for a certain purpose, and reabsorbed later on. Schindler[a] and Tschirch[b] both elaborated on the hypothesis of Brunchhorst, Tschirch going so far as to classify the "bacteroids" with the vegetable caseins.

"As I have shown," wrote Marshal Ward in 1887,[c] "this hypothesis is utterly untenable with regard to the tubercles on the roots of *Vicia faba*, and everything points to its being equally so for the other leguminosæ; not only is Tschirch's attempt to explain away the hyphæ and the gemules a failure, but his drawings indicate that he is not in possession of the histological facts necessary to constitute him an authority on the subject of the development and physiology of the root tubercles of the leguminosæ, whence his repeated assumptions lose in value."

In discussing his own experiments, Ward says:[d]

> The chief points to notice are: (1) The all but invariable development of the tubercles within a month, when the beans were germinated in sand or soil not previously heated; (2) their nondevelopment when the medium was sterilized by being heated; (3) the number of times I succeeded in infecting the roots by means of pieces of old tubercles placed among the root hairs; and (4) the number of times the infecting hypha was discovered entering the cortex by means of the root hairs.
>
> It is partly on these grounds that I infer that the tubercles so common on the roots of the bean are due to the action of the fungus, the very minute germs of which are all but universally distributed in the soil.

When this statement of Ward's appeared in print, Hellriegel[e] had already announced that he had found the legume tubercles to be due to bacterial infection, and that this infection was beneficial, rather than harmful, to the host plant, since it was the means of providing the latter with combined nitrogen. He was inclined to regard the tubercles as laboratories where the formation of nitrogen compounds out of atmospheric nitrogen took place. In a later publication he and Wilfarth[f] showed that normal plants of the legume family could be grown in artificial soils containing but traces of combined nitrogen, but provided with the mineral elements of plant food. However, the development was normal only when tubercles were formed on the roots. Without the tubercles the plants lacked the ability to utilize atmospheric nitrogen for their growth. No tubercles were formed in the sterilized soil, and the plants in the latter died of nitrogen hunger. When, however, such sterilized soil was treated with some fresh soil infusion, the formation of tubercles

[a] Jour. Landw., 33 (1885), p. 325.
[b] Ber. Deut. Bot. Gesell., 5 (1887), p. 58.
[c] Phil. Trans. Roy. Soc. London, Ser. B, 178 (1887), p. 556.
[d] Ibid., p. 557.
[e] Tagebl. 59. Versamml. Deut. Naturf. u. Aerzte, Berlin, 1886, No. 7, p. 290.
[f] Ztschr. Ver. Deut. Rübenz. Indus., Beilageheft, Nov. 1888.

took place and the plants grew normally. Hellriegel and Wilfarth's statements soon received ample confirmation in several directions. Tschirch,[a] who was apparently the first to attempt the cultivation of the "bacteroids" on artificial media, failed to obtain positive results. Beijerinck [b] was more successful, and named the organism isolated by him *Bacillus radicicola,* although the fact that the bacteria-like bodies in the tubercles were true bacteria had already been demonstrated by Wigand.[c] Further confirmation of Hellriegel and Wilfarth's claim was furnished by the thorough and painstaking investigations of Prazmowski.[d] He demonstrated that the hypha-like bodies in the tubercles were filled with bacteria, which passing into the cell plasma were transformed into bacteroids. Also, Prazmowski isolated cultures of *B. radicicola* on artificial media, viz, nutrient gelatin of variable composition. The tubercle organism was also grown on artificial media or otherwise studied by Frank,[e] Gonnermann,[f] Mazé,[g] Stutzer,[h] Hiltner and his associates,[i] Morck,[j] Neumann,[k] Steglich,[l] Smith,[m] Buhlert,[n] and a host of others. Mention should be made in this connection of the exact investigations of Schloesing and Laurent,[o] who demonstrated the fixation of atmospheric nitrogen by the joint activities of leguminous plants and *Bacillus* (*Ps.*) *radicicola*, by the actual diminution of the amount of elementary nitrogen in the inclosed atmosphere surrounding the plants. It was thus demonstrated that the soil-enriching quality of leguminous vegetation was due to its ability to utilize atmospheric nitrogen for its growth, and that this ability was imparted to it by the bacteria living in symbiosis with it.

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[a] Ber. Deut. Bot. Gesell., 5 (1887), p. 58.

[b] Bot. Ztg., 46 (1888), pp. 725, 741, 757, 781, 797.

[c] Bot. Hefte, 2 (1887), p. 88.

[d] Bot. Centbl., 36 (1888), pp. 215, 248, 280; Bul. Internat. Acad. Sci. Cracovie, 1889, No. 6, p. XXVIII; Landw. Vers. Stat., 37 (1890), p. 161; 38 (1891), p. 5.

[e] Ber. Deut. Bot. Gesell., 7 (1889), p. 332; Landw. Jahrb., 19 (1890), p. 523.

[f] Landw. Jahrb., 23 (1894), p. 649.

[g] Les Microbes des Nodosités des Legumineuses. Thesis, Sceaux, 1898.

[h] Mitt. Landw. Inst. K. Univ. Breslau, 1900, No. 3, p. 57.

[i] Centbl. Bakt. [etc.], 2. Abt., 6 (1900), p. 273; Landw. Vers. Stat., 39 (1891), p. 327; 45 (1895), pp. 1, 155; 47 (1896), p. 257; 49 (1898), p. 467; 51 (1899), p. 447; Arb. Biol. Abt. Land u. Forstw. K. Gsndhtsamt., 1 (1900), p. 177; 3 (1903), pp. 151, 445.

[j] Über die Formen der Bakteroiden bei den einzelnen Species der Leguminosen. Inaug. Diss., Leipzig, 1891.

[k] Landw. Vers. Stat., 56 (1902), p. 187.

[l] Ber. Tät. Landw. Abt., K. Vers. Stat. Pflanzenkult. Dresden, 1903.

[m] Proc. Linn. Soc. N. S. Wales, 1901, p. 152.

[n] Centbl. Bakt. [etc.], 2. Abt., 9 (1902), pp. 148, 226, 273, 892; Fühling's Landw. Ztg., 51 (1902), p. 385.

[o] Ann. Inst. Pasteur, 6 (1892), pp. 65, 824.

Space would not allow a further discussion here of the very numerous contributions on the subject of symbiotic nitrogen fixation.[a]

[a] See Atwater, W. O., and Woods, C. D. Amer. Chem. Jour., 12 (1890), No. 8, p. 526; 13 (1891), No. 1, p. 42.
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[List continued on next page.]

They have been reviewed thoroughly by Jacobitz[a] and Vogel,[b] to whom the writers are indebted for a number of references. We can not forego the attempt, however, to consider here some of the views on the character of symbiotic nitrogen-fixation, and particularly the far-reaching theoretical conclusions that will, no doubt, serve as a fruitful stimulus to future practice.

It is scarcely necessary to point out here that the amounts of nitrogen gathered by leguminous crops in a single season may be quite large. The experiments conducted by the New Jersey stations[c] demonstrated the value in this direction of crimson clover, which was found to have added more than 200 pounds of nitrogen per acre, and similar studies at the Delaware Station[d] showed the yields of nitrogen to range from 139 to 188 pounds per acre. Experiments with velvet beans showed nitrogen gains amounting to 213 pounds per acre in Alabama, 172 pounds in Louisiana, and 141 pounds in Florida.[e] Like results were obtained with other legumes showing in the United States an average gain for sixteen States of 122 pounds per acre.[f]

These enormous amounts of nitrogen are withdrawn from the atmosphere by the joint activities of the leguminous plants and of the bacteria within their tubercles, and the term symbiosis used to designate these joint activities is, everything considered, not inappropriate. To be sure, Fischer,[g] and after him Moore,[h] endeavored to prove that the relation of *Pseudomonas radicicola* to the host plant represents a case of parasitism pure and simple, yet in the very definition of the term, parasitism involves a more or less marked injury to the host, which, at best, derives no benefit from the invading organism. In the phenomenon under consideration, however, there is, under normal conditions, a distinct gain to the host plant, and hence the term "parasitism" is not applicable.

[Continued from previous page.]

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[b] Ibid., 15 (1905), pp. 33, 174, 215.

[c] New Jersey Stas. Rpt. 1894, p. 158.

[d] Delaware Sta. Bul. 67.

[e] Florida Sta. Bul. 60, p. 456.

[f] Moore. U. S. Dept. Agr., Bur. Plant Indus. Bul. 71, p. 19.

[g] Vorlesungen über Bakterien. Jena, 1903.

[h] U. S. Dept. Agr., Bur. Plant Indus. Bul. 71, p. 35.

The exact nature of these symbiotic relations is not yet clearly understood. Hiltner would explain them by his "immunity" theory, which is, in substance, as follows:[a] Active tubercles immunize the plant against bacteria of an equal or lower degree of virulence than is possessed by those already within the tubercles; only bacteria of a higher degree of virulence may still gain entrance into the roots. Hiltner would thus eliminate the influence of the host plant itself and ascribe the immunization to the substances formed by the bacteroids within the tubercles. In support of his theory he refers to the fact that alder plants in nitrogen-free solutions, whose tubercles contain sufficient bacteria, show no further infection on the newly formed roots, notwithstanding the repeated inoculations which at once caused infection in similar plants free from tubercles.

Süchting,[b] on the other hand, would explain these relations by his more simple and also more plausible "equilibrium" theory. He notes the analogy in the invasion of the legume roots by *P. radicicola* on the one hand, and of the animal body by pathogenic bacteria on the other. There is seemingly a production in either case of toxins by the invading organisms, and of antibodies on the part of the organisms invaded, with the difference noted above, that the legume succeeds in deriving a benefit from the invasion of its roots by *P. radicicola*. Furthermore, vigorous plants, like vigorous animals, resist more strongly the activities of the invading organisms by their greater power of neutralizing the toxins, and hence would yield to the invasion of highly virulent bacteria only. There is thus established in the formation of tubercles a state of equilibrium between the antibodies of the plant and the infection bodies (toxins) of the bacteria. Hiltner's observation with alder plants could just as easily be accounted for by Süchting's theory in that the failure of the already infected alder plants to form new tubercles was due to their greater resisting power to infection acquired through the nitrogen compounds elaborated in their root tubercles. On the other hand, those alder plants which possessed no tubercles at all were weakened by nitrogen hunger, and hence could not resist the invasion of *P. radicicola*. Süchting would also explain the well-known fact that fewer tubercles are formed by legumes when growing in media containing nitrates by the assumption that the better nitrogen feeding enhances the resisting power of the plants.

The equilibrium relations as outlined by Süchting involve, then, varying conditions of virulence on the part of the bacteria, and varying resisting power on the part of the higher plants. As to

[a] Arb. Biol. Abt. K. Gsndhtsamt., 1 (1900), p. 177; see also Centbl. Bakt. [etc.], 2. Abt., 11 (1904), p. 387.

[b] Centbl. Bakt. [etc.], 2. Abt., 11 (1904), p. 417.

variations in virulence, his own experiments are a source of much interesting information on the subject. In comparing the efficiency of pure cultures, of emulsions from crushed tubercles, and of soil, he found the first two decidedly superior to the last-named in influencing nitrogen fixation by the plants. It was clearly demonstrated, therefore, that variations in virulence do exist. Moreover, a comparison of plants inoculated with material from crushed tubercles and from pure cultures showed the latter to be equal or superior to the former, provided suitable media were used. On the other hand, it was shown that unsuitable media reduced the virulence of the bacteria, and, therefore, the nitrogen fixation by the plants. That the virulence of the bacteria may be maintained on artificial media for a long time was shown by an experimental comparison in which cultures kept on artificial media for a year proved as efficient as the inoculating material from freshly crushed tubercles. Mention should also be made here of the very interesting observation by Süchting that the vegetation period of the plant varies, with the virulence of the inoculating material, in that increasing virulence runs parallel with a shortening of the vegetation period.[a]

As to the agents of nitrogen fixation, this much may be said: It is generally believed now that the direct agents of fixation are the bacteria, and that the plant utilizes the nitrogen compounds elaborated by them. But in this utilization of the nitrogen compounds the host plant may either attack and absorb the highly nitrogenous bacteroids or it may avail itself of the soluble and diffusible nitrogenous substances elaborated within the bacterial cell. To be sure, there is room here for difference of opinion, yet, as Süchting points out,[b] no proteolytic ferments have so far been detected in the legumes, and it would hardly be justifiable, therefore, to take it for granted that the plants secure their nitrogen by dissolving and absorbing the bacteroids. It is all the more difficult to understand for this reason why Moore[c] maintains that "the cells of the roots are able to secrete an enzym which dissolves the nodule organism when in the branched condition, and by this method renders available considerable quantities of nitrogen, which is then diffused through the plant."

He further elaborates on this by saying:

That the bacteria are almost always able to resist the action of the host plant, except when in the branched condition, is undoubtedly true, although there are a few exceptions in the case of the pea and one or two other plants. If the only source of nitrogen is by dissolving the bacteria, it will readily be seen that should the nodules continue to be filled with the unbranched rods, the benefit to the plant will be little or nothing, and the presence of nodules upon the roots may even be a

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[a] Centbl. Bakt. [etc.], 2. Abt., 11 (1904), p. 517.

[b] Ibid., p. 501.

[c] U. S. Dept. Agr., Bur. Plant Indus. Bul. 71, p. 33.

detriment. Too little attention has been paid to this point, the almost universal opinion being that all nodules are able to supply nitrogen to the plant, and any failure in a crop well supplied with these growths must be due to other causes. This is not the fact, however, there being no question that frequently the organisms producing nodules have lost the power of going into the branched condition, and thus, while preventing their destruction by the plant, they defeat the very object for which they are supposed to be so valuable. That this condition is due to the organism itself, and is not the result of lack of vigor on the part of the plant which prevents its secreting the enzym that will make the bacteria available, is proved by the fact that it is possible to control this situation by modifying the character of the bacteria. Thus, if nodule-forming organisms be grown upon artificial media for a long time, where they are almost invariably in the rod condition, this form becomes so firmly established that plants inoculated with such cultures, although forming nodules, receive practically no benefit, the nodules remaining firm and hard, and furnishing no nitrogen to the roots.[a]

The contention that nodule-forming organisms grown upon artificial media for a long time may have the rod form so very firmly established as to make the material unsuitable for inoculation purposes does not appear to be well established. It has been noted that under proper conditions bacteria grown upon artificial media for a year may lead to as much or more nitrogen fixation than may be due to emulsion from freshly crushed tubercles, and there is sufficient evidence to show that the formation of rods or of bacteroid forms depends more on culture conditions than heredity. Indeed, Süchting believes he has proved that bacteroid formation is determined by the concentration in the medium of the metabolism products of *P. radicicola*.[b] The almost invariable absence of bacteroids on solid media, and their more frequent occurrence in liquid media, may be quite plausibly accounted for by the fact that there is a greater concentration of the products of metabolism on the former, and, therefore, the suppression of bacteroid formation. This view is intimately connected with another theory on the mechanism of nitrogen assimilation, a theory more in harmony with the experimental data thus far accumulated.

It has been shown that *P. radicicola* is capable of fixing atmospheric nitrogen, but evidently in much smaller quantity than it is capable of fixing in the legume tubercles. The difference could possibly be accounted for by many hypotheses, yet there is one that is not merely simple, but is also strengthened by experimental evidence. It is that the nitrogen is fixed within the bacterial cells, that the nitrogen compounds thus formed are soluble and diffusible through the cell wall, and that the nitrogen compounds thus diffusing outward are rapidly taken up by the host plant. One of the writers has demonstrated that in the fixation of nitrogen by *Bacillus pyocyaneus*,[c] as

[a] U. S. Dept. Agr., Bur. Plant Indus. Bul. 71, p. 34.

[b] Centbl. Bakt. [etc.], 2. Abt., 11 (1904), p. 381.

[c] New Jersey Stas. Rpt. 1902, p. 229.

well as by members of the Azotobacter group,[a] there is the formation of nitrogen compounds not precipitated by basic lead acetate, and that cultures of *Azotobacter vinelandii* passed through an unglazed porcelain filter still contain considerable quantities of combined nitrogen. It is not at all unreasonable to suppose, therefore, that the nitrogen compounds formed within the bacterial cells are diffusible. Indeed, Moore supplies testimony to this effect, since his cultures of *P. radicicola* passed through a Pasteur-Chamberland filter still contained a small percentage of combined nitrogen.[b] This soluble and diffusible nitrogen compound, or compounds, is used by the bacteria for further syntheses, and as insoluble protein substance becomes a part of the bacterial body. Under normal conditions there can evidently be no large accumulation of the soluble nitrogen compound in the culture medium, for it is used up almost as fast as it is formed. Applying this reasoning to the elucidation of the processes within the legume tubercles, it would seem that the nitrogen compound diffused from the bacterial cells is rapidly appropriated by the plant. The bacteria are thus forced to increase the output of combined nitrogen, so as to supply enough building material for their own development. Modifications occur in the bacterial cells that would allow the maximum elaboration of combined nitrogen, and the bacteroids come into being. It is possible that bacteroid formation could also be forced in artificial cultures, accompanied by intensive fixation of atmospheric nitrogen, if means were provided for the gradual and constant removal of the diffusible nitrogen compound in question. Süchting's view as to the nonformation of bacteroids on solid media would be supported by such a demonstration, and the latter would then go to prove also that the concentration of such nitrogen compounds determines the extent of nitrogen fixation, both within and without the legume tubercles. Such a demonstration would likewise make clear Neumann's[c] observation corroborated by Süchting[d]—that in older liquid media the number of bacteroids is considerably diminished by the division of the latter into the small motile forms—and would be in line with Nobbe and Hiltner's statement that there is an intimate relation between nitrogen fixation by legumes and bacteroid formation within their tubercles in that the beginning of fixation is coincident with the appearance of bacteroids[e] in the nodules, and that the plants apparently unable to utilize atmospheric nitrogen contain few bacteroids, or none at all, within their tubercles. There appears to be also some connection

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[a] New Jersey Stas. Rpts. 1904, p. 270; 1905, p. 254.

[b] U. S. Dept. Agr., Bur. Plant Indus. Bul. 71, p. 33.

[c] Landw. Vers. Stat., 56 (1901), p. 187.

[d] Centbl. Bakt. [etc.], 2. Abt., 11 (1904), p. 384.

[e] Stefan. Centbl. Bakt. [etc.], 2. Abt., 16 (1906), p. 148.

between glycogen formation and nitrogen fixation by *P. radicicola*, Azotobacter species, and other organisms.[a]

The assumption that the nitrogen feeding of legumes is directly dependent on the amount of combined nitrogen elaborated by their bacteria would lead to the logical conclusion that most nitrogen would be fixed under conditions most favorable for the rapid development and increase of the bacteria. Such a conclusion could not be accepted, however, without reservation. It would be at variance with the fact that at times the host plant derives no benefit from its bacteria, even though the latter be found to grow vigorously in its nodules. In order to account for this peculiarity, Süchting offers a very ingenious and very plausible explanation.[b] He distinguishes between virulence and vegetative power, the former not necessarily a function of the latter. In the case of *P. radicicola* the virulence is measured by its nitrogen-fixing power. Strongly virulent organisms possess this power in a very pronounced degree, while nonvirulent cultures, the so-called pseudo forms, are entirely devoid of this power. It may thus happen that while there is a rapid multiplication of the bacteria within the nodules there is still no accompanying fixation of nitrogen. Under such conditions *P. radicicola* may become a parasite in the true sense of the word, taking nonnitrogenous and perhaps also nitrogenous substances from the plant juices and giving nothing in return. Different combinations also could thus be made of highly virulent cultures possessing a high vegetative power, of moderately virulent cultures with a high vegetative power, of nonvirulent cultures with a high vegetative power, of moderately virulent cultures with a low vegetative power, etc. It follows therefore that there must be a stage in the relation of virulence to vegetative power that is most advantageous to the host plant, since it is quite evident that the nonnitrogenous materials withdrawn by the bacteria from the plant are more or less of a drain upon its resources and must be more than made good by the nitrogen compounds supplied by them. Quite conceivably cultures with a high vegetative power and moderate or low virulence leave a balance on the debit side, and the plant would be weakened rather than strengthened by their presence. This may even apply to cultures possessing a high vegetative power and a high degree of virulence. It would thus become possible to explain the repeated observation that plants inoculated with highly virulent cultures produce a lower yield than similar uninoculated plants. The relation of tubercle formation to the nitrate supplied would also be easier to understand, since the soluble nitrogen compound, by increasing the vigor of the plants,

[a] Heinze. Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 76.
[b] Centbl. Bakt. [etc.], 2. Abt., 11 (1904), p. 502.

would preclude the entrance of any but highly virulent organisms, and there might be a rough relation between the amount of nitrate applied and the degree of virulence of the entering organisms.

The views discussed in the preceding pages, bordering at times on speculation, possess a deep significance for future soil bacteriological research, and are treated here in detail because of their importance in the elucidation of obscure phenomena. The subject can not be dismissed, however, without a reference at least to a rather unique hypothesis of Hiltner's.[a] He maintains that no nitrification occurs in soils where legumes are growing vigorously and are fixing large quantities of nitrogen. The soluble nitrogen compounds already present in the soil are transformed by certain other bacteria into insoluble combinations, the bacteria in the soil itself thus cooperating with the plant when within the spheres of the root activities, which he designates as "rhizospheres." The free nitrogen-fixing bacteria within the rhizospheres are also stimulated in their activities by the plant roots. Thicker seeding brings the rhizospheres nearer to one another and increases nitrogen fixation. It is of practical significance to determine to what extent the inoculation of the soil with the bacteria which transform soluble nitrogen compounds into insoluble modifications enhance the activities of the nitrogen-fixing bacteria. In commenting on this hypothesis Löhnis[b] is inclined to admit that there may be some connection between the consumption of nitrates by *P. radicicola*, *Azotobacter chroococcum*, and other nitrogen-fixing bacteria and their power of nitrogen fixation.

SOIL INOCULATION.

Agricultural literature records many attempts at soil inoculation—that is, the introduction into the soil of micro-organisms that would increase its crop-producing power. By force of circumstances these attempts are practically all centered about a single phase in the great range of soil-fertility problems, namely, that of nitrogen fixation. A review of the work accomplished will show that such attempts dealt either with symbiotic or nonsymbiotic nitrogen-fixing bacteria, or those supposed to be capable of fixing atmospheric nitrogen.

Something has already been said under the head of Alinit of the work of Caron and of the striking results obtained by Stoklasa, unfortunately not substantiated by other investigators. The matter is regarded as having been settled some years ago, and nothing further need be said about it in this place. Still other attempts at soil inoculation with nonsymbiotic nitrogen-fixing bacteria have been carried out with members of the Azotobacter group, and the results

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[a] Arb. Deut. Landw. Gesell., 1904, No. 98, p. 59; abs. in Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 46.

[b] Centbl. Bakt. [etc.], 2. Abt., 14 (1905), p. 714.

thus far have been purely negative. Gerlach and Vogel[a] were the first to experiment in this direction, inoculating either the seed or soil, but could detect no gain in the inoculated plats that could clearly be attributed to Azotobacter. The addition of glucose, glycerin, straw, sodium lactate, and similar substances depressed the yield very considerably. Negative results with Azotobacter inoculation were also obtained by Freudenreich[b] who, like Gerlach and Vogel, used cultures of *A. chroococcum*. Experiments by one of the writers[c] with *A. vinelandii* and *A. beijerincki* likewise yielded negative results. On the whole, however, such work can only be regarded as preliminary. At the same time it does not follow that means may not be found in the future to control the nitrogen fixation in the soil of the nonsymbiotic nitrogen-gathering bacteria. The very fact that under certain conditions, at present unexplained, these organisms may cause a very marked increase in the store of soil nitrogen, as has already been noted, makes it desirable that a better understanding of these conditions be secured, and that such knowledge be intelligently directed in the service of agriculture. Practical methods of promoting the activity of nitrogen-fixing organisms in the soil are being studied especially by Koch.[d]

A greater measure of achievement, as well as of immediate promise, may be found in the record of soil inoculation with symbiotic nitrogen-fixing bacteria. In looking through this record one encounters at the outset a question not yet fully settled after a decade and a half of research and discussion. This question is of vital importance, both theoretically and practically in the growth of legumes, for it concerns the very nature of the tubercle organisms. Is *P. radicicola* (*Bacillus radicicola*), first isolated by Beijerinck,[e] one and the same in all legumes, or are there different species, or merely different varieties of it? Beijerinck himself found great similarity in the colonies from the different legumes, but later found more decided differences.[f]

Hellriegel,[g] who obtained an increased yield of peas, vetches, beans, and clover when he treated them with soil leachings from a field that had grown peas and various clovers, but not serradella and lupines, could get no increase when he applied these leachings to serradella and lupine plants, and was led to conclude that there were essential differences between the bacteria from different legumes. Nobbe,

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[a] Centbl. Bakt. [etc.], 2. Abt., 9 (1902), p. 881.
[b] Ibid., 10 (1903), p. 514.
[c] New Jersey Stas. Rpt. 1904, p. 279.
[d] Mitt. Deut. Landw. Gesell., 21 (1906), pp. 111; 22 (1907), p. 117.
[e] Bot. Ztg., 46 (1888), pp. 725, 741, 757, 781, 797.
[f] Ibid., 48 (1890), p. 837.
[g] Ztschr. Ver. Deut. Rübenz. Indus., Beilageheft, Nov., 1888.

Schmid, Hiltner, and Hotter[a] in a manner confirmed Hellriegel's observations by demonstrating wide differences between the organism from plants of the Robinia and Pisum genera, yet could not decide whether these were different species, varieties, or simply different cultural conditions. Extensive experiments by the four first named led them to assert in 1894,[b] that all of the tubercle inhabitants of the different legumes examined by them, even of the Mimosaceæ, belong to one species, *Bacillus radicicola* Beijerinck, but that the latter is so strongly affected by the roots in which it develops that its progeny retains full efficiency only for the legume species to which the host plant belongs, and loses it in greater or slighter degree for all others. They conclude that since the tubercle bacteria may multiply also outside of the plant, it must be supposed that they are universally present also in soils which had not borne legumes for a long time, and in which, therefore, there could not have occurred an accommodation to any particular species. The universal occurrence of such, in a manner neutral, soil bacteria would undoubtedly account for the experience that the seeding of legumes in places where they had never grown before is followed by tubercle formation.

The views thus promulgated by these authors left a deep impression and found their way into a number of text-books. The experiments of Nobbe and Hiltner, reported in the following year,[c] cast some doubt, at least, on the accuracy of these sweeping conclusions. The experiments in question proved that certain results from inoculation are obtained only when the plants are inoculated with tubercle bacteria of the same plant species.[d] More recently Hiltner found himself obliged to admit[e] that no positive experimental proof has as yet been furnished to show that tubercle bacteria of widely removed plant species may replace one another, and believes himself justified in dividing the tubercle bacteria into two groups, one of them including the organisms of Lupinus, Ornithopus, and Soja, and designated as *Rhizobium beijerinckii;* the other including those of Pisum, Vicia, Lathyrus, Phaseolus, Trifolium, Medicago, Anthyllis, Onobrychis, and Robinia. Süchting, who critically examined Hiltner's classification, is not inclined to agree with him, holding that the extremely difficult question as to the specific identity of the tubercle bacteria is far from settled.[f]

Moore[g] is rather more positive in his views than Süchting, and on

[a] Landw. Vers. Stat., 39 (1891), pp. 327–359.

[b] Ibid., 45 (1894), p. 25.

[c] Ibid., 47 (1896), p. 257.

[d] Ibid., p. 266.

[e] Süchting. Centbl. Bakt. [etc.], 2. Abt., 11 (1904), p. 379.

[f] Ibid., p. 380; Schultze, Centbl. Bakt. [etc.], 2. Abt., 10 (1903), p. 665.

[g] U. S. Dept. Agr., Bur. Plant Indus. Bul. 71, p. 25.

the strength of extensive experiments, but incompletely reported, he says:

> It is satisfactorily demonstrated that it is possible to cause the formation of nodules upon practically all legumes, no matter what the source of the original organisms, provided they were cultivated for some time upon a synthetic nitrogen-free medium.
>
> It is undoubtedly true that the long adaptation of the bacteria to the special conditions obtaining in a particular species of legume enables such organisms to produce more abundant nodules in shorter lengths of time than bacteria isolated from some other legume and grown upon nitrogen-free media. While this is of considerable practical importance, and will probably always make it necessary to distribute the specific organism for the specific crop, it does not in any way indicate that the bacteria found in the nodules of beans, peas, clovers, etc., are separate species. The most that can be maintained is that there is a slight physiological difference due to the long association with a plant of a peculiar reaction which enables the bacteria more easily to penetrate the host upon which they have been accustomed to grow. These slight racial characteristics can readily be broken down by cultivation in the laboratory, and it is entirely possible to secure a universal organism capable of producing a limited number of nodules upon all legumes which now possess these growths.[a]

Moore thus comes back to Nobbe, Hiltner, and Schmid's views of 1895, and his neutralization of *P. radicicola* on artificial culture media finds an analogy in the supposed neutralization of this organism in soils kept free from leguminous plants, as stated by these authors.[b] But, whatever the theoretical status of the matter, there is also a practical significance in it that was recognized more than a decade and a half ago. Some years before pure cultures of *P. radicicola* came into more general use attempts were made to practically apply Hellriegel and Wilfarth's discovery by the employment of old legume soil as inoculating material on newly reclaimed lands or on lands where the corresponding legume had not been grown previously.

As early as 1887 inoculation experiments with old legume soils were initiated by Salfeld[c] at the Moor Culture Experiment Station, at Bremen. As the season advanced Salfeld noticed the wonderful effect of the inoculations on the reclaimed moor soils in the dark green color and luxuriant growth of the inoculated plants, whereas the field where no legume soil had been scattered bore very small yellow plants that ultimately succumbed to nitrogen hunger. He also noticed that the two legume soils derived from different sources and used for inoculation did not benefit the plants to an equal extent. The results of these experiments were published in 1888, and were received with great interest in agricultural circles, according to Schmitter,[d] who says that the first attempt at soil inoculation,

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[a] U. S. Dept. Agr., Bur. Plant Indus. Bul. 71, p. 26.

[b] For recent studies on the identity of the root tubercle organism of leguminous plants, see Maassen and Müller. Mitt. K. Biol. Anst. Landw. u. Forstw. 1907, No. 4, p. 42. Rossi, G., Centbl. Bakt. [etc.], 18 (1907), pp. 289, 481.

[c] Deut. Landw. Presse, 15 (1888), No. 99, p. 630.

[d] Schmitter, A. Die Impfung des Lehmbodens zu Lupinen mit bakterienreicher Erde. Inaug. Diss., Heidelberg [Erfurt], 1892.

according to Hellriegel's method, proved successful under actual field conditions. The practical results furnished a great stimulus, especially for the national development of moor soils. Schmitter questions whether this would apply to other soils and conditions, and proceeds to discuss the results secured from soil inoculation up to his time (1892). He mentions Salfeld's experiments in 1888 and 1889, Fleischer's experiments in 1891, Hansen's in 1890, and Fruwirth's in 1891, the experiments of Arnstadt, and numerous experiments on farms and estates, besides his own experiments carried on in 1889, 1890, and 1891. Briefly stated, the results secured by himself and his contemporaries showed plainly that on moor soils recently placed under cultivation, on sandy heath soils where no legumes had been previously grown, on raw soils brought up from deeper layers by the plow, and on soils that had been burned the results of soil inoculation with material from old legume fields were quite satisfactory, and often startling. Moreover, a number of instances were recorded also where such inoculations produced favorable results on loam or clay loam soils, although most of the experiments with these soils gave negative results. Reviewing the experiments made up to 1892, Schmitter says that the results show distinctly that the opinions concerning this question are quite contradictory. On the one hand were reports of successful inoculation, on the other were experiments no less reliable in which the results were negative. The successful practice of soil inoculation still presented some difficulties. Only in one instance had it been clearly of practical value, and that was in the case of raw soils. On ordinary loam and clay soils that had been subjected to rational methods of farming the success of such inoculations was a matter of doubt, depending not merely upon the inoculation itself, but also upon other factors which called for further study.

In the early nineties of the last century the number of field inoculation experiments was quite considerable. We might note here the observation recorded by Salfeld in 1892, that soils from under different legume crops could not be used interchangeably for the inoculation of any particular legume. For instance, on newly reclaimed soils a crop of peas benefited greatly from inoculation with soil from an old pea field, whereas soil taken from a lupine field failed to yield like results.

Here, then, was a practical demonstration of the peculiar effects of soil inoculation corroborated also by the experience of Fruwirth,[a] and a demonstration, likewise, of the dissimilarity between lupine and pea soil as inoculating material. As time went on the limitations of this method of soil inoculation were felt more strongly. It was rather expensive to transport large quantities of soil through

[a] Deut. Landw. Presse, 19 (1892), Nos. 1, p. 6; 2, p. 14.

long distances, considerable labor was involved in its distribution, and the danger was frequently present of introducing objectionable weeds, fungus diseases, etc. These considerations made it desirable that a method involving the use of pure cultures be introduced, and, accordingly, there was placed on the market about the middle of the nineties the bacterial preparation "Nitragin," a pure culture on nutrient gelatin of *P. radicicola*, put up by a commercial firm of Höchst, Germany. Each legume had its corresponding culture, a practical recognition of the actual or possible differences in bacteria from different sources.

Nitragin was extensively tested[a] in Europe and in this country, with results not at all satisfactory. Official reports in Germany for the years 1896 and 1897, embracing the work of agricultural stations or institutes at Bonn, Marburg, Göttingen, Hildesheim, Halle, Breslau, Proskau, Köslin, Dahme, Berlin, and the moor-culture station of the Ems division, showed partially favorable returns from only four of these.[b] Negative results were obtained also in many of the American tests[c] of Nitragin, including those at the New Jersey Station. From these experiments Frank concludes that:

It is possible to enhance the growth of legumes by inoculating the soil with artificially grown legume bacteria, such as are contained in Nitragin, but that something is lacking in this artificial preparation that is present in the natural cultures of legume bacteria, and it is to this lack that the many failures of Nitragin inoculation should be attributed.[d]

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[a] See Remy. Verhandl. Gesell. Deut. Naturf. u. Aerzte [Karlsbad], 74 (1902), I, p. 200.
Hiltner. Arb. Biol. Abt. Land- u. Forstw. K. Gsndhtsamt., 3 (1902), p. 1.
Paratore. Sul polomorfismo del *Bacillus radicicola* Beij. Malpighia, 15 (1901), p. 175.
Remy. Deut. Landw. Presse, 29 (1902), pp. 31, 37, 46.
Nobbe and Richter. Landw. Vers. Stat., 56 (1902), p. 441; 59 (1904), p. 167.
Hiltner. Deut. Landw. Presse, 29 (1902), p. 119.
Marchal. Compt. Rend. Acad. Sci. [Paris], 133 (1901), p. 1032.
Wohltmann and Bergené. Jour. Landw., 50 (1902), p. 377.
Hiltner. Bericht über die Ergebnisse der im Jahre 1903 in Bayern ausgeführten Impfversuche mit Reinculturen von Leguminosenknöllchen Bacterien (Nitragin). Stuttgart, 1904. Prakt. Bl. Pflanzenbau u. Schutz, 2 (1904), pp. 13, 43, 69; Naturw. Ztschr. Land- u. Forstw., 2 (1904), p. 127.
Schneider. Prakt. Bl. Pflanzenbau u. Schutz, 1 (1903), p. 25.
Thiele. Ztschr. Landw. Kammer Provinz Schlesien, 1902, No. 43, p. 1311.
Salfeld. Illus. Landw. Ztg., 24 (1904), No. 13, p. 133.

[b] Frank. Landw. Vers. Stat., 51 (1899), p. 441.

[c] See New Jersey Stas. Rpt. 1899, p. 367.
Maine Sta. Rpts. 1897, p. 144; 1898, p. 208.
Massachusetts Hatch. Sta. Rpt. 1897, p. 26.
Dawson, Maria. Ann. Bot., 15 (1901), p. 511.
U. S. Dept. Agr., Farmers' Buls. 65, p. 19; 124, p. 7.

[d] Landw. Vers. Stat., 51 (1899), p. 444.

Frank was inclined to account for the diminished efficiency of Nitragin by the unsuitable media (gelatin) upon which the organisms were grown. He observes that the natural medium, where these bacteria live, is the soil and the living plant cell, but that gelatin is a substance that differs very materially from this medium, and the suspicion is not excluded that the efficiency of the bacteria may undergo a change with the changed conditions of growth. He suggested, therefore, an experimental comparison of various media to determine whether there might not be among them some that would enable the bacteria to retain their natural efficiency for the legumes and yet remain suitable from the commercial standpoint.

Nobbe and Hiltner, the originators of Nitragin, were not willing to see in the numerous failures a convincing argument against artificial inoculation, expressing a desire to learn from just such failures in what direction Nitragin itself and the method of its application may be capable of improvement. They believed that if it were possible to increase or diminish at pleasure the virulence of the bacteria there must come a turning point in the estimate of Nitragin, for an inoculation with bacteria whose virulence was artificially increased would not only assure success in every case, but would also benefit such soils as had previously but feebly reacted to Nitragin, because of their content of more or less efficient bacteria.[a]

In working out these ideas they introduced very essential modifications in the preparation and application of Nitragin. The early method of dissolving the gelatin which carried the culture in pure water was changed when it was realized that variations in osmotic pressure might produce plasmolysis and cause the death of the organisms, and it was proposed to use either milk or a salt solution instead of pure water. A comparison of gelatin and liquid media showed also that the latter were better adapted to maintain the vitality of the organisms for a longer time, and while Nobbe and Hiltner could not decide, for various reasons, to dispense with the gelatin entirely, they still continued to experiment with different media. The gradual improvement of the cultures was followed by fruitful results, and in 1903 Hiltner was able to report a decided success in 60 out of every 100 inoculations in 180 experiments by farmers and institutions.[b] In the year 1903 there were carried out in Bavaria 98 inoculation experiments, of which 81 were favorable, 9 negative, and 8 doubtful—a very good showing, indeed, since an increased yield from pure cultures was also obtained on some soils that had previously borne good crops of the corresponding legume.[c]

The use of pure cultures for soil inoculation was extensively tried by the United States Department of Agriculture in 1904. The

[a] Landw. Vers. Stat., 51 (1899), p. 451.

[b] 5. Internat. Kong. Angew. Chem. [Berlin], 1903, Ber. 3, p. 799.

[c] Centbl. Bakt. [etc.], 2. Abt., 12 (1904), p. 497.

method employed was rather different from that followed in connection with Nitragin, and, as developed by Moore,[a] involved the use of liquid cultures and of absorbent cotton. The latter was dipped in the culture of *P. radicicola*, made in nitrogen-poor media to increase their virulence, dried quickly at a low temperature, and subsequently used as inoculating material in the preparation of other liquid cultures. The cotton cultures and accompanying nutrient salts were distributed free to the farmers of the United States. Moore explains the purpose of this free distribution as follows:[b]

In order that the bacteria might have the most thorough practical test possible, the Department of Agriculture has for the last year conducted one of the largest experiments of its nature ever undertaken in any country. By the free and unlimited distribution of cultures to practically every one who was sufficiently interested to request a package, it has been possible to secure about 12,500 tests under the most varied conditions in almost every State of the Union.

A definite increase of crop directly attributable to inoculation was noted in 1,296 out of 2,502 reports.

Tests have been made of cultures prepared according to Moore's method by a number of experiment stations in the United States[c] and also in Europe,[d] with negative results in many cases. Tests by the New York State Station, substantially confirmed by the work of several other stations, have shown the commercial nitrocultures prepared according to the Moore method to be as a rule inefficient, and indicate that the difficulty is inherent in the method of preparing the cultures.

In the water-culture experiments carried out by Remy,[e] the plants inoculated with nitroculture failed to produce tubercles, whereas similar plants inoculated with Nitragin, under the same conditions, produced an abundance of tubercles on their roots. Similarly, in the case of pot experiments, where either pasteurized sand or soil was used, the nitroculture proved wholly ineffective, while under the same conditions the Nitragin was found to be quite efficient. Remy attributes the loss of efficiency to the drying of the cultures in preparation, and concludes that success from the use of the nitro-

[a] U. S. Dept. Agr., Bur. Plant Indus. Bul. 71, p. 37.

[b] Ibid., p. 43.

[c] See Georgia Sta. Bul. 71; Kentucky Sta. Bul. 125; Maine Sta. Bul. 128; Massachusetts Sta. Rpt. 1905, p. 77; New York Cornell Sta. Bul. 237; New York Sta. Buls. 270, 282; Oklahoma Sta. Bul. 68; Pennsylvania Sta. Bul. 78; Texas Sta. Bul. 83; Virginia Sta. Bul. 159; West Virginia Sta. Bul. 105; Wisconsin Sta. Rpt. 1905, p. 242; Canada Expt. Farms Rpt. 1905, p. 130; Ontario Agr. Col. and Expt. Farm Bul. 148; Harding, Science, n. ser., 25 (1906), p. 122; Harding, Syllabi of Lectures, Normal Institute held at New York Agricultural Experiment Station, Nov. 26–Dec. 1, 1906.

[d] Ber. Tät. K. Landw. Chem. Vers. Stat. Wien, 1905, p. 101; Centbl. Bakt. [etc.], 2. Abt., 17 (1906), p. 660.

[e] Centbl. Bakt. [etc.], 2. Abt., 17 (1906), p. 660.

cultures for the inoculation of legumes is, for this reason, not to be expected.

A. F. Woods,[a] of the United States Department of Agriculture, attributes many of the reported failures with nitrocultures prepared by the Moore method to lack of skill and care required to make and keep the cultures in good condition, two essentials often disregarded being the necessity for quick drying of the cotton carrying the culture and protection of the dried culture from moisture until used. In view of the difficulties which have been encountered with dry-cotton cultures the Department has substituted for them, and is now distributing, nitrogen-free liquid cultures in hermetically sealed receptacles.

There is sufficient experimental evidence to prove beyond doubt that the use of pure cultures for soil inoculation is quite practicable. Yet uncertainty and the danger of failure are almost always present in the practical use of such cultures. Leaving out of consideration such soils as are not adapted to the survival of the legume bacteria, or soils that are so poor in potash, phosphoric acid, or other plant-food elements that nitrogen is no longer the minimum factor there, there is grave danger of failure even in soils that are in themselves adapted to yield increased crops on inoculation.[b] This danger is due to inefficient culture material or the improper application of efficient culture material. As to the first, it need only be pointed out that carelessness, indifference, and ignorance on the part of those engaged in the commercial preparation of such cultures may easily enough lead to a rapid deterioration of originally efficient cultures. Frequent checking of the purity of such cultures, frequent reisolation, frequent change of the culture media, and careful regard for virulence are absolutely indispensable if they are to be maintained in a high state of efficiency.

As to the danger of improper treatment of efficient inoculating material, it should be remembered that the nutrient salts employed in the preparation of liquid media offer splendid culture material for a host of other bacteria and yeasts. The directions, for instance, of one of the commercial companies in the United States call for the use of clean water for the solution of the salts. Contamination here is not excluded whether this clean water come from the well or cistern, and the contaminating organisms frequently develop very rapidly and injure or entirely suppress the legume bacteria. And thus it happens often enough that the farmer inoculates his seed with *Bacillus megatherium*, or with various yeast cells, instead of *P. radicicola*.

It may be said in view of the foregoing that there is more or less uncertainty at present as to the method to be followed in inoculating soil with *P. radicicola*. While generally recognizing the need of inoc-

[a] U. S. Dept. Agr. Yearbook 1906, p. 135.

[b] For study of conditions favoring legume inoculation, see Kellerman, K. F., and Robinson, T. R., U. S. Dept. Agr., Bur. Plant Indus. Bul. 100, pt. 8, and Farmers' Bul. 240.

ulation, under certain conditions, a number of the experiment stations advise the use of soil for this purpose as is done, for instance, by the Maine,[a] the New York (Cornell),[b] and Illinois[c] stations. In a recent bulletin of the New York (Cornell) Station it is stated in the case of alfalfa that "outside of the districts where alfalfa growing is already well established only 6 per cent of the cooperative tests indicate no need of efforts to secure inoculation. In 63 per cent of the cases no nodules at all were found. Certainly New York farmers must look well after the matter of inoculation if they would secure success with alfalfa, for without the alfalfa organism the crop does not succeed in New York." We are told further that "extended experiments and observations lead to the conclusion that where inoculation is needed, and the conditions are favorable for the bacteria, the use of soil from an old alfalfa field uniformly results in abundance of nodules." In the Maine Experiment Station bulletin just referred to it is stated that "if one desires to grow alfalfa, soy beans, cowpeas, or other leguminous plants that are not usually grown in the State, the inoculation by the application of soil from a field that has grown the desired legume with an abundance of root tubercles is the only sure way yet devised. This inoculation, by the transfer of soil carrying the organism, has never given negative results so far as the writers know."

UNSOLVED PROBLEMS.

The broad problems of future research in soil bacteriology must concern, first of all, the methods by means of which the bacteriological factor in soil fertility may be determined in any particular case. The beginning made in this direction by Remy and others has already been noted. It remains now to develop these methods and to amplify them so as to render their use widely practicable. At the same time it would be idle to expect that bacteriological data in themselves, no matter how complete, will ever be sufficient for a clear interpretation of fertility conditions. Such an interpretation, if attempted, would be as one sided as that based on purely chemical, or purely mechanical analytical data. There must be a coordination here in the three fields of research and a knowledge sufficiently broad to allow a proper recognition and an exact measurement of the essential factors. The mean summer temperature, the water-holding or capillary power of a soil, its permeability to gases, its content of soluble salts, the geological origin of its constituent particles, the proportion and nature of its organic matter—each and all of these exert a marked influence on the bacterial flora, and the latter in turn affects the humus and soluble salt content and through these soil reactions and soil fertility.

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[a] Maine Sta. Bul. 126, p. 28. [b] New York (Cornell) Sta. Bul. 237, p. 144.
[c] Illinois Sta. Bul. 94.

Theoretically, at least, there is for each soil a condition of highest bacterial efficiency, a maximum ammonification, a maximum nitrification, and a maximum nitrogen fixation, accompanied by a minimum evolution of free nitrogen, and a minimum conversion of soluble into insoluble nitrogen compounds. It will be the task of the future bacteriologist to ascertain the proportion of humus most suitable for any soil type, and to attempt with the aid of the chemist to modify the nature of this humus to provide for a maximum bacteriological efficiency. It will probably be found that different soil types will need different bacterial combinations, in order to make a maximum efficiency possible in every case. It is likely enough that by changing the reaction of the soil, or the proportion and composition of its organic matter, we shall learn to shift the bacterial equilibrium in the direction desired, just as treatment with carbon bisulphid, ether, chloroform, etc., enabled Hiltner and Richter to change the normal flora of their soils. It may even be that we shall learn to develop in the laboratory strains of ammonifying or of nitrifying bacteria of such high bacteriological efficiency as to produce by their inoculation into the soil a marked and beneficial influence on soil fertility. It is quite probable that we shall learn to increase our store of soil nitrogen by intensifying the activities of the nonsymbiotic nitrogen-fixing bacteria, either through soil improvement, soil inoculation, or both. There is ample promise that soil inoculation with legume bacteria will be rendered more practicable, more remunerative, and therefore more extensive. We shall undoubtedly extend our studies of the many bacterial species in the soil, we shall learn to know the products of their metabolism when growing in pure cultures and the modification of these products through associative action, and the influence of such modification on crop production.

Future research in soil bacteriology will teach us how the bacterial flora of the soil is affected by crop rotation; it will teach us more as to to the influence of various crops on the number and kind of prevailing bacterial species. We shall learn to know how fertilizers and various combinations of fertilizers, of organic manures and combinations of manures and mineral fertilizers, affect the quantity and quality of the soil bacteria. We shall extend our soil-bacteriological studies to the flora of irrigated lands and shall surely reap a rich harvest there of varied knowledge and shall derive thence many an instructive lesson to be turned to good account in the economy of crop production.

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